Calcasieu Estuary Remedial Investigation/Feasibility Study (RI/FS): Baseline Ecological Risk Assessment (BERA)

Appendix E2: Assessment of Risks to the Benthic Invertebrate Community in the Calcasieu Estuary

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Prepared For:

CDM Federal Programs Corporation
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Dallas, Texas 75231

Under Contract To:

Mr. John Meyer, Regional Project Manager
U.S. Environmental Protection Agency, Region 6
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Prepared – October 2002 – By:

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In Association With:

United States Geological Survey
4200 New Haven Road
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Table of Contents

List of Tables

List of Figures

Appendix E2. Assessment of Risks to the Benthic Invertebrate Community in the Calcasieu Estuary

1.0 Introduction

In response to concerns regarding environmental contamination in the Calcasieu Estuary, a Remedial Investigation/Feasibility Study (RI/FS) is being conducted in the estuary. One of the objectives of the RI/FS is to assess the risks posed by environmental contamination to ecological receptors that inhabit key areas of the Calcasieu Estuary. To meet this objective, a baseline ecological risk assessment (BERA) must be undertaken in accordance with the procedures laid out by the United States Environmental Protection Agency (USEPA) in the *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments* (USEPA 1997a). Under the eight-step process described by the USEPA for conducting a BERA, a screening ecological risk assessment (SERA) must be conducted to provide preliminary estimates of exposure and risk.

In 1999, CDM Federal Programs Corporation (CDM) conducted a SERA for the Calcasieu Estuary which concluded that there was a potential risk to ecological receptors inhabiting the estuary from exposure to contaminated sediment and/or surface water (CDM 1999). In September 2001, a Baseline Problem Formulation (BPF; Appendix A; MacDonald *et al.* 2001) was prepared that identified chemicals of potential concern (COPCs) and areas of interest, described the environmental fate and ecological effects of the COPCs, and identified key exposure pathways and receptors at risk in the Estuary. The BPF also led to the development of assessment and measurement endpoints, a conceptual model and a risk analysis plan for the

BERA. Accordingly, the BPF defined the issues that needed to be addressed in the BERA for the Calcasieu Estuary.

One of the important conclusions of the BPF was that benthic invertebrate communities are likely to be exposed to various COPCs that occur in whole sediments and pore water. The other groups of aquatic receptors that are addressed in the Calcasieu Estuary BERA include: microbial communities (Appendix C); aquatic plant communities (Appendix D); fish communities (Appendix F1 and F2); avian communities (Appendix H); and, mammalian communities (Appendix I). The COPCs in whole sediments, surface water, and/or pore water that were identified in the BPF included various metals (i.e., copper, chromium, lead, mercury, nickel, and zinc), chlorinated ethanes (i.e., 1,2-dichloroethane and trichloroethane; DCE and TCA), polycyclic aromatic hydrocarbons (PAHs; i.e., 13 parent PAHs and total PAHs), polychlorinated biphenyls (PCBs; i.e., various Aroclor mixtures, PCB congeners, and total PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans [PCDFs; i.e., expressed as tetrachlorodibenzo-*p*-dioxins - toxic equivalents (TCDD-TEQs)], hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), bis(2-ethylhexyl)phthalate (BEHP), carbon disulfide, unionized ammonia, hydrogen sulfide, acetone, and several organochlorine pesticides (i.e., aldrin and dieldrin; see Table A1-7 of Appendix A1).

1.1 Conceptual Model

The conceptual site model represents a particularly important component of the problem formulation because it enhances the level of understanding regarding the relationships between human activities and ecological receptors at the site under consideration. Specifically, the conceptual site model describes key relationships between stressors and assessment endpoints. In so doing, the conceptual model provides a framework for predicting effects on ecological receptors and a template for generating risk questions and testable hypotheses (USEPA 1997a; 1998). The conceptual site model also provides a means of highlighting what is known and what is not known about a site. In this way, the conceptual model provides a basis for identifying data gaps and designing monitoring programs to acquire the information necessary to complete the assessment. Conceptual site models consist of two main elements:

- A set of hypotheses that describe predicted relationships between stressors, exposures, and assessment endpoint responses (along with a rationale for their selection); and,
- Diagrams that illustrate the relationships presented in the risk hypotheses.

The conceptual site model of the Calcasieu Estuary is described in Chapter 7 of the BPF (Appendix A). More specifically, that chapter summarizes the available information on the sources and releases of COPCs, the fate and transport of these substances, the pathways by which ecological receptors are exposed to the COPCs, and the potential effects of these substances on the ecological receptors that occur in the Calcasieu Estuary. In turn, this information was used to develop a series of hypotheses that provide predictions regarding how ecological receptors are exposed to and respond to the COPCs. The conceptual site model, which describes the exposure pathways of greatest interest for benthic invertebrate communities, was adopted for use in this deterministic risk assessment. Based on the pathways identified in the conceptual model, whole sediments and pore water are likely to represent the most important routes of exposure to COPCs for the benthic invertebrate community. For this reason, other possible exposure pathways (e.g., surface water) were not evaluated relative to the potential for adverse effects on the benthic invertebrate communities in the estuary.

1.2 Areas of Concern

The Calcasieu River is one of the largest river systems in southwest Louisiana (LA). From its headwaters in the vicinity of Kisatchie National Forest (in Vernon Parish), the Calcasieu River flows some 260 km to the Gulf of Mexico near Cameron, LA (Figure E2-1). While much of the Calcasieu River system is relatively uncontaminated, the portion of the watershed from the saltwater barrier near Lake Charles, LA to the Intercoastal Waterway has undergone extensive industrial development over the past five decades. These developmental activities have resulted in widespread contamination in the estuarine portion of the watershed, particularly in the bayous within the upper portion of the estuary (Curry *et al.* 1997).

In response to public concerns, USEPA is conducting a federally-led RI/FS to assess risks to human health and ecological receptors and to evaluate remedial options for addressing environmental contamination in the Calcasieu Estuary. Based on the results of the SERA, the portion of the Calcasieu Estuary from the saltwater barrier to Moss Lake was identified as the area in which environmental contamination posed the greatest potential risks to ecological receptors and, as such, was designated as the primary study area (CDM 1999). To facilitate the RI/FS, this study area was divided into four sub-areas (termed Areas of Concern; AOC), including:

- Upper Calcasieu River AOC (UCR AOC);
- Bayou Verdine AOC (BV AOC);
- Bayou d’Inde AOC (BI AOC); and,

- Middle Calcasieu River AOC (MCR AOC).

Several reference areas were also identified in the lower estuary and in the vicinity of Sabine National Wildlife Refuge to support the interpretation of the data generated during the RI. As a BERA for the Bayou Verdine has already been completed by Conoco, Inc. and Condea Vista (Entrix, Inc. 2001), ecological risks in the BV AOC were not assessed in this report.

1.3 Chemicals of Potential Concern

The identification of COPCs represents an essential element of the problem formulation process (USEPA 1998). To initiate this process, CDM conducted a SERA of the Calcasieu Estuary in 1999 to assess the potential for adverse biological effects on ecological receptors associated with either direct or indirect exposure to contaminated environmental media in the Calcasieu Estuary (CDM 1999). To support this assessment, historical data on the levels of environmental contaminants in surface water, sediment, and biota were collated and compiled (CDM 1999). Subsequently, the maximum measured concentration of each substance in each media type was compared to the lowest ecological screening value for that substance to facilitate the determination of maximum hazard quotients. These maximum hazard quotients provided a basis for identifying the substances in surface water, sediment, and biota of the estuary that occurred at levels sufficient to potentially affect one or more ecological receptors. These substances were termed chemicals of potential concern (COPCs) in the Calcasieu Estuary and included: metals; PAHs; PCBs; organochlorine and other pesticides; chlorophenols; chlorinated benzenes; chlorinated ethanes; phthalates; cyanide; and, acetone.

Because the preliminary list of COPCs that emerged from the SERA contained over 100 substances (CDM 1999), it was determined that it required further refinement to assure that only those substances with a relatively high probability of adversely affecting ecological receptors were addressed in further investigations. For this reason, a scoping meeting was convened in Denver, Colorado (CO) in July, 2000 to develop a more focused list of COPCs. The scoping meeting was attended by risk assessors, risk managers, and the USEPA Region VI Ecological Technical Assistance Group (ETAG). Rather than relying on historical data (as was done in the SERA), the participants at this scoping meeting used the results of the Phase I sampling program of the RI to identify the COPCs in the Calcasieu Estuary (Goldberg 2001). For water-borne contaminants, the substances that occurred in unfiltered water samples at total concentrations in excess of the ambient water quality criteria (WQC; i.e., criteria continuous concentrations; CCCs; USEPA 1999a) were deemed to be COPCs. For sediment-associated constituents, the substances that occurred in whole sediments at concentrations in excess of the effects range median values (ERMs; Long *et al.* 1995) or comparable sediment quality benchmarks (e.g., probable effect levels PELs; MacDonald *et al.* 1996; CCME 1999) were considered to be COPCs. Based on the results of these evaluations, the scoping meeting participants agreed that the following substances were the primary COPCs in the Calcasieu Estuary:

Water-Borne COPCs

- Metals (copper and mercury);
- 1,2-dichloroethane; and,
- Trichloroethane.

Sediment-Associated COPCs

- Metals (copper, chromium, lead, mercury, nickel, and zinc);

- Polycyclic aromatic hydrocarbons (PAHs; acenaphthene, acenaphthylene, anthracene, fluorene, 2-methylnaphthalene, naphthalene, phenanthrene, benz[a]anthracene, benzo(a)pyrene, chrysene, dibenz[a,h]anthracene, fluoranthene, pyrene, total PAHs, and other PAHs);
- Polychlorinated biphenyls (PCBs);
- Polychlorinated dibenzo-*p*-dioxins (PCDDs), and, polychlorinated dibenzofurans (PCDFs);
- Chlorinated benzenes [hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), and degradation products];
- Phthalates [bis(2-ethylhexyl)phthalate (BEHP)];
- Carbon disulfide;
- Unionized ammonia;
- Hydrogen sulfide;
- Acetone; and,
- Organochlorine pesticides (aldrin and dieldrin).

Because exposure to whole sediments and pore water represents the principal routes through which the benthic invertebrate community can be exposed to toxic chemicals, all of the water-borne and sediment-associated substances were identified as COPCs relative to the benthic invertebrate community.

1.4 Purpose of Appendix

The purpose of this appendix is to provide an evaluation of the risks posed to benthic invertebrate communities associated with exposure to COPCs in the Calcasieu Estuary. Benthic invertebrates are the animals that live in and on the sediments in freshwater and estuarine ecosystems. Benthic animals are extremely diverse and are represented by nearly all taxonomic groups from protozoa to large invertebrates. The groups of organisms that are commonly associated with benthic communities include protozoa, sponges (i.e., Porifera), coelenterates (such as *Hydra* sp.), flatworms (i.e., Platyhelminthes), bryozoans, aquatic worms (i.e., oligochaetes), crustaceans (such as mysids, decapods, and amphipods), mollusks (such as oysters and clams), and aquatic insects (such as dragonflies, mayflies, true flies, and aquatic beetles). Because benthic invertebrate communities are difficult to study in a comprehensive manner, benthic ecologists often focus on the relatively large members of benthic invertebrate communities, which are known as benthic macroinvertebrates. These organisms are usually operationally defined, for example, as those that are retained on a 0.5 mm sieve. Benthic invertebrates represent key elements of aquatic food webs because they consume aquatic plants (i.e., such as algae and aquatic macrophytes) and detritus. In this way, these organisms facilitate transfer of energy and nutrients to fish, birds, and other organisms that consume aquatic invertebrates.

There are a number of studies that have been conducted to evaluate the composition of benthic macroinvertebrate communities in the Calcasieu Estuary. For example, Gaston (1987a) collected sediment samples from 28 stations to evaluate the structure of macroinvertebrate communities in the Calcasieu River/Lake complex during 1983 and 1984. The results of this investigation indicated that surface deposit feeders and sub-surface deposit feeders accounted for more than 75% of the total abundance of benthic macroinvertebrates in the upper estuary. The polychaetes, *Streblospio benedicti*, *Hobsonia florida, Laeonereis culveri, Polydora socialis, Nereis succinea, Parandalia fauveli* and *Polydora ligni*, were the most abundant surface deposit feeders in the upper estuary (i.e., from the headwaters to the outlet of Prien Lake). Sub-surface deposit feeders and suspension feeders were also observed in the upper estuary, including oligochaetes (e.g., Tubificidae and Naididae), polychaetes (e.g., *Mediomastus californiensis*), gastropods (e.g., Mactridae; probably *Rangia cuneata*), midges and amphipods (e.g., *Corophium louisianum*; Gaston 1987a; Gaston and Nasci 1988; Gaston *et al.* 1988).

The benthic macroinvertebrate community in the middle portion of the estuary (i.e., from the outlet of Prien Lake to the head of Calcasieu Lake) was similar to that in the upper estuary. However, surface deposit feeders and suspension feeders represented the two main trophic groups in the middle estuary, collectively accounting for more than 70% of the total abundance of macroinvertebrates (Gaston and Nasci 1988). The surface deposit feeders were largely the same as those observed in the upper estuary. The principal suspension feeders included the amphipods, *Corophium louisianum* and *Corophium lacustre*, and *Hargeria rapax* (Tanaidacea). During the summer and fall, sub-surface deposit feeders, primarily oligochaetes (i.e., Tubificidae) and polychaetes (e.g., *Mediomastus californiensis*), were present at the highest densities (Gaston 1987a; 1987b).

In the lower estuary (i.e., Calcasieu Lake), the benthic invertebrate community was typically dominated by sub-surface deposit feeders, which comprised more than 60% of the total abundance of macroinvertebrates in this area (Gaston and Nasci 1988). The sub-surface deposit feeders in the lower estuary were primarily polychaetes, such as *Mediomastus californiensis* and *Capitella capitata*, and oligochaetes (i.e., Tubificidae). Surface deposit feeders and suspension feeders comprised the majority of the other benthic macroinvertebrates that were observed in this area; these included polychaetes (*Streblospio benedicti*, *Hobsonia florida, Nereis succinea, Paraprionospio pinnata, Parandalia fauveli,* and *Polydora ligni),* mysids *(Mysidopsis* sp.*),* amphipods (e.g., *Cerapus benthophilis* and *Corophium louisianum*), bivalves (i.e., Mactridae), and *Hargeria rapax* (Tanaidacea). Isopods *(Edotea triloba)* were also observed in the lower estuary (Gaston 1987a; 1987b).

To date, the ecological assessment work conducted for the Calcasieu Estuary has not characterized the potential risks to the benthic invertebrate community associated with exposure to COPCs in environmental media. Risk hypotheses laid out in the BPF indicate that many COPCs pose a potential risk to the benthic invertebrate community from direct contact with contaminated sediments and/or pore water. This appendix provides an evaluation of the risks posed to the benthic invertebrate community associated with exposure to the COPCs in the Calcasieu Estuary.

2.0 Methods

A step-wise approach was used to assess the risks to the benthic invertebrate community posed by exposure to COPCs in the Calcasieu Estuary. The five main steps in this process included:

- Identification of assessment endpoints, risk questions and testable hypotheses, and measurement endpoints;
- Collection, evaluation, and compilation of the relevant information on sediment quality conditions in the Calcasieu Estuary;
- Assessment of the exposure of benthic invertebrates to COPCs (i.e., exposure assessment);
- Assessment of the effects of COPCs on benthic invertebrates (i.e., effects assessment); and,

- Characterization of risks to the benthic invertebrate community (i.e., risk characterization).

Each of these steps is described in the following sections of this appendix.

2.1 Identification of Assessment Endpoints, Risk Questions, and Measurement Endpoints

An assessment endpoint is an 'explicit expression of the environmental value that is to be protected' (USEPA 1997a). The selection of assessment endpoints is an essential element of the overall environmental risk assessment (ERA) process because it provides a means of focusing assessment activities on the key environmental values (e.g., reproduction of sediment-probing birds) that could be adversely affected by exposure to environmental contaminants. Assessment endpoints must be selected based on the ecosystems, communities, and species that occur, have historically occurred, or could potentially occur at the site (USEPA 1997a).

A measurement endpoint is defined as 'a measurable ecological characteristic that is related to the valued characteristic that is selected as the assessment endpoint' and it is a measure of biological effects (e.g., mortality, reproduction, growth; USEPA 1997a). Measurement endpoints are frequently numerical expressions of observations (e.g., toxicity test results, community diversity measures) that can be compared to similar observations at a control and/or reference site. Such statistical comparisons provide a basis for evaluating the effects that are associated with exposure to a contaminant or group of contaminants at the site under consideration.

To support the identification of key assessment and measurement endpoints for the Calcasieu Estuary BERA, the USEPA convened a BERA workshop in Lake Charles, LA on September 6 and 7, 2000. The workshop participants included representatives of the USEPA, United States Geological Survey (USGS), National Oceanic and Atmospheric Administration (NOAA), Louisiana Department of Environmental Quality (LDEQ), United States Fish and Wildlife Service (USFWS) and CDM (hereafter termed the Calcasieu Estuary Ecological Risk Assessment Advisory Group). The workshop was explicitly designed to enable participants to articulate the goals and objectives for the ecosystem (i.e., based on the input that had been provided by the community in a series of public meetings), to assess the status of the knowledge base, to define key issues and concerns, and to identify the COPCs and AOCs in the study area. Importantly, this workshop provided a basis for refining the candidate assessment endpoints that had been proposed based on the results of the SERA (CDM 1999). Additionally, workshop participants identified a suite of measurement endpoints that would provide the information needed for evaluating the status of the assessment endpoints (MacDonald *et al.* 2000a).

To be effective, the measurement endpoints must be linked to the assessment endpoints by a series of risk questions. These risk questions, which can be restated as testable hypotheses, describe the specific assumptions about the potential risk to assessment endpoints posed by exposure to COPCs in environmental media. The risk questions were developed using a combination of professional judgement and information on the potential sources of stressors, stressor characteristics, and actual and predicted ecological effects on the selected assessment endpoints (USEPA 1998). The conceptual model diagrams presented in the BPF provide a visual representation of the risk hypotheses.

2.2 Collection, Evaluation, and Compilation of Relevant Information on Sediment Quality Conditions in the Calcasieu Estuary

Information on the chemical and toxicological characteristics of whole sediments and/or pore water were collected in two phases, including Phase I and Phase II sampling programs. The methods that were used to collect the samples, quantify the levels of COPCs in various media types, evaluate the toxicity of sediment samples, evaluate the reliability of the resultant data, and compile the information in a form that would support the BERA are described in the following sections.

Sample Collection - The RI of the Calcasieu Estuary was conducted in two phases. In Phase I of the investigation, more than 500 sediment samples were collected at sites located throughout the estuary between November, 1999 and March, 2000, based on a stratified random sampling design. The samples collected during this phase of the sampling program were intended to provide the data needed to assess the nature and extent of contamination within the estuary. The Phase II sampling program was designed to augment the information that was collected in Phase I by providing further information on the nature, severity and areal extent of contamination, assessing the bioavailability of environmental contaminants, evaluating the effects on ecological receptors associated with exposure to contaminants, and filling outstanding data gaps. In Phase II of the investigation, more than 100 whole-sediment samples and 50 pore-water samples were obtained, again based on a stratified random sampling design. The locations of the sampling sites in the UCR/BV AOCs, BI AOC, MCR AOC, and reference areas are shown in Figures E2-2, E2-3, E2-4a, E2-4b, and E2-5, respectively.

As indicated above, a stratified random sampling design was utilized in both phases of the RI. More specifically, the estuary was divided into five areas (i.e., UCR AOC, BV AOC, BI AOC, MCR AOC, and reference areas; Figure E2-1), multiple reaches within each area, and numerous sub-reaches within each reach. Subsequently, the number of samples that were to be collected within each area, reach, and sub-reach was determined based on the size of the area, historic contamination patterns, and other factors. Then, the USEPA Region V Fully Integrated Environmental Location Decision Support (FIELDS) tools were used to randomly select coordinates (i.e., latitude and longitude) for the assigned number of primary sampling stations and alternate sampling stations (i.e., which would sampled if it was not possible to obtain samples from any primary sampling stations). In the field, each sampling station was located with the aid of navigation charts and a Trimble differentially-corrected global positioning system (GPS). Sediment samples were collected within a 10 meter radius of the designated coordinates. If it was not possible to collect a sample within the 10 meter radius of the coordinates (e.g., the water was too deep, substrate was gravel, etc.), then a previously selected alternate station was sampled.

The methods that were used to collect, handle, and transport the sediment samples collected in the Phase I and Phase II sampling programs are described in CDM (2000a; 2000b; 2000c; 2000d; 2000e). Briefly, surficial sediment samples (i.e., the top 10 cm) were collected with a modified large Eckman dredge (23 x 23 cm). Deeper sediment samples were collected with a piston sampler. At each station, multiple grab samples (up to 10 grabs) were obtained, composited, and homogenized to support the preparation of splits for various chemical analyses, pore-water extraction, and/or toxicity tests. In accordance with the methods described in CDM (2000e), pore-water samples were obtained by pneumatic squeezing of whole sediments at the Marine Environmental Research Center (USGS) in Corpus Christi, Texas. All whole-sediment and pore-water samples were shipped to laboratories in plastic coolers on ice.

Chemical Analyses - Chemical analysis of the sediment samples collected during the Phase I and Phase II sampling programs was conducted at various contract laboratory program (CLP) and subcontract (non-CLP) analytical laboratories, including Quanterra-Severn Trent Laboratories, USEPA Region VI Laboratory, USEPA Region VI CLP laboratories, Olin Contract laboratories, and PPG Industries contract laboratories. Upon receipt at the laboratory, sediment and pore-water samples were held at 4° C in the dark until selection for analysis.

In Phase I, total metals in whole sediments were quantified using a variety of analytical methods, including E6020, SW6010B, SW7471A, and SW6020. Polycyclic aromatic hydrocarbons and/or other semi-volatile organic compounds (SVOCs) were quantified using one or more of the following methods: SW8260B; SW8270C; HOU-SVOC; SW8015B; and, SW8015B MOD. Method SW8260B was used to quantify volatile organic compounds. Polychlorinated biphenyls were quantified using SW8081A, while pesticides and herbicides were quantified using SW8081A and SW8151A, respectively. Finally, PCDDs and PCDFs were measured using SW8290. A summary of the analyses that were conducted at each analytical laboratory is presented in Table E2-1.

In Phase II, total and total recoverable metals were analyzed in whole sediments by the USGS Columbia Environmental Research Center (CERC) using inductively-coupled plasma-mass spectrometry (ICP-MS; May *et al.* 1997). Methods SW6010B, SW7471A, 200.7, and 200.9 were used by American

Analytical and Technical Services Inc. (AATS) and USEPA Region VI Laboratory to quantify the concentrations of total recoverable metals in whole-sediment samples. Simultaneously extracted metal (SEM) and acid volatile sulfide (AVS) concentrations were quantified by USGS-CERC using the procedures outlined in USEPA (1991). Total and methyl mercury were analyzed by Texas A&M University using methods SW7470A, SW7471B, and USGS 005. Polycyclic aromatic hydrocarbons and other semi-volatile organic compounds were measured by AATS and USEPA Region VI Laboratory using SW 8270C, while PCBs Aroclors were measured by AATS and USEPA Region VI Laboratory using SW8082. Polychlorinated biphenyl congeners were analyzed by ALTA Laboratories using SW1668, while PCDDs and PCDFs were analyzed by ALTA Laboratories using SW8290.

The concentrations of COPCs in pore-water samples collected in the Phase II sampling program were determined by three laboratories. The USGS Columbia Environmental Research Laboratory conducted analyses of total and dissolved metals in pore water using inductively-coupled plasma-mass spectrometry (ICP-MS; May *et al.* 1997). Method 8270C-SIM, SW8270C, and gas chromatography-mass spectrometry (GCMS) were used by AATS and Texas A&M University to measure the levels of PAHs in pore water, while SW8081A and SW8081 were used by AATS to quantify PCB (Aroclors) concentrations. Method SW8081A and gas chromatography-electron capture device (GC-ECD) were used by AATS and Texas A&M University to measure pesticide concentrations. Finally, congener analysis of PCBs was conducted by Texas A&M University using GC-ECD.

Whole-Sediment Toxicity - The results of whole-sediment toxicity tests were used to evaluate the effects of contaminated sediments on the survival of benthic invertebrates. More specifically, the results of 10-day whole-sediment toxicity tests with the infaunal amphipod, *Ampelisca abdita*, were used to evaluate the effects of contaminated sediments on the survival of benthic invertebrates (ASTM 2001b; Harding ESE, Inc. 2001). In addition, the effects of sediment-associated contaminants on invertebrate survival or growth were evaluated using the results of 10-d and 28-d whole-sediment toxicity tests with the epibenthic amphipod, *Hyalella azteca* (ASTM 2001a; 2001b; USEPA 2000a; MESL 2001).

Whole-sediment toxicity tests were conducted on 100 whole-sediment samples collected during the Phase II sampling program. The survival of amphipods exposed to Calcasieu Estuary sediments was compared with that of amphipods exposed to reference sediments from the study area. Sediment samples were designated as toxic if amphipod survival in Calcasieu Estuary sediments was lower than the lower limit of the normal range (i.e., 95% confidence interval) for the reference sediments ($p<0.05$; i.e., using a reference envelope approach). The normal range of responses of amphipods exposed to reference sediments was determined by calculating the 2.5^{th} and 97.5^{th} percentiles of the survival data (i.e., following data transformation to achieve normality). Although several other procedures could have been used to designate samples as toxic or not toxic (e.g., analysis of variance (ANOVA) compared to control, paired T-tests with control results, minimum significant difference from control; Thursby *et al.* 1997), the reference envelope approach was utilized because it provides a means of evaluating incremental toxicity at test sites when compared to reference sites (Hunt *et al.* 2001). In this way, only the toxicity that is attributable to differences in the characteristics of test and reference samples is considered for the purposes of the BERA. That is, the reference envelope approach provides a basis of determining the toxicity that is attributable primarily to COPC-related factors.

The results of whole-sediment toxicity tests were also used to evaluate the effects of contaminated sediments on the growth of the benthic invertebrates. More specifically, the effects of sediment-associated contaminants on invertebrate growth were evaluated using the results of 10-d and 28-d whole-sediment toxicity tests with *Hyalella azteca* (ASTM 2001a; USEPA 2000a; Ingersoll *et al.* 2001). The growth of amphipods exposed to Calcasieu Estuary sediments was compared with that of amphipods exposed to reference sediments from the study area. Sediment samples were designated as toxic if amphipod growth in Calcasieu Estuary sediments was lower than the lower limit of the normal range (i.e., 95% confidence interval) for the reference sediments ($p<0.05$). The results of the 10-d and 28-d *Hyalella azteca* tests were similar; however, the results of the 28-d test were somewhat less variable and were in better agreement with a previously developed database for relationships between sediment chemistry and toxicity (Appendix E1; USEPA 2000b). Therefore, the results of the 28-d test rather than the 10-d test with *Hyalella azteca* were used in the BERA.

Pore-Water Toxicity - The effects of sediment-associated contaminants on the reproduction of invertebrates were evaluated using the results of pore-water toxicity tests. More specifically, the effects of contaminated sediments on invertebrate reproduction was evaluated using the results of pore-water toxicity tests with the sea urchin, *Arbacia punctulata*, in which fertilization and embryo development were measured. In this context, the sea urchin fertilization and embryo development test were used as surrogates for reproductive effects on other invertebrate species (Carr and Chapman 1992; Carr *et al.* 1996a; 1996b;

2001). Pore-water toxicity tests were conducted on 50 sediment samples collected during the Phase II sampling program. The fertilization success and embryo development of sea urchins exposed to pore water from Calcasieu Estuary sediments were compared with that of sea urchin gametes and embryos exposed to pore water from reference sediments from the study area. Pore-water samples were designated as toxic if fertilization or embryo development in pore water from Calcasieu Estuary sediments was lower than the lower limit the normal range (i.e., 95% confidence interval) for the reference sediments ($p<0.05$).

Benthic Invertebrate Community Assessment - The effects of contaminated sediments on benthic invertebrates were evaluated using the results of benthic invertebrate community structure analyses. More specifically, data on five key indicators of benthic invertebrate community structure, including the abundance of pollution indicator species, the abundance of pollution sensitive species, species richness, total abundance, and a normalized macroinvertebrate index of biotic integrity (mIBI) were used to evaluate impacts on benthic invertebrate community structure in the estuary. Appendix E3 provides a description of the procedures used to calculate the mIBI. These metrics were selected because the results of previous studies have demonstrated they provide an effective basis for identifying sediment samples with degraded benthic communities (Gaston and Nasci 1988; Gaston *et al.* 1988; Gaston and Young 1992; Brown *et al.* 2000).

The original goal outlined in the BPF (MacDonald *et al.* 2001) was to compare the five benthic invertebrate community metrics for Calcasieu Estuary sites to the normal range of these metrics for reference sites (i.e., the upper or lower 95% confidence intervals; Reynoldson *et al.* 1995). However, the variability in these metrics was too high for the reference sites to calculate 95% confidence intervals that could be used to evaluate the response of benthic invertebrates in the Calcasieu Estuary sites. For this reason, the data for all sites were evaluated together, using cluster analysis, to identify impacted and unimpacted samples (Ingersoll *et al.* 1997; SAS 2001). When the benthos samples were clustered in groups, it was observed that one cluster of 52 samples contained sites with the lowest average benthic metrics and highest average chemical concentrations in sediment compared to sites in the remaining nine clusters. Therefore, sediment samples in this first cluster were designated as having an impacted benthic invertebrate community relative to the samples in the remaining nine clusters (i.e., toxic conditions for the benthic invertebrate community).

Data Validation and Verification - All of the data sets that were generated during the course of the study were critically reviewed to determine their applicability to the assessment of risks to the benthic invertebrate community in the Calcasieu Estuary. The first step in this process involved validation of all of the sediment and pore-water chemistry data. Following translation of these data and the biological effects data into database format, the validated data were then further evaluated to assure the quality of the data used in the risk assessment. This auditing process involved analyses of outliers (i.e., to identify inconsistencies with units) and completeness (i.e., to identify missing samples or missing data); examination of data qualifier fields (i.e., to assure internal consistency in the BERA database), checking of sample identification numbers (i.e., to ensure that data were not duplicated). Finally, the data were fully verified against the original data source.

Database Development - To support the compilation and subsequent analysis of the information on sediment quality conditions in the Calcasieu Estuary, a relational project database was developed in Microsoft Access format. All of the sediment chemistry, pore-water chemistry, benthic invertebrate community structure, and sediment toxicity data compiled in the database were georeferenced to facilitate mapping and spatial analysis using geographic information system (GIS)-based applications [i.e., Environmental Systems Research Institute, Inc. (ESRI's) ArcView and Spatial Analyst programs]. The database structure made it possible to retrieve data in several ways, including by data type (i.e., chemistry vs. toxicity), by sediment horizon (i.e., surficial vs. sub-surface sediments), by stream reach (i.e., Upper Bayou d'Inde vs. Lower Bayou d'Inde), by sub-reach (i.e., Upper Bayou d'Inde-1 vs. Upper Bayou d'Inde-2), and by date (i.e., Phase I vs. Phase II). As such, the database facilitated a variety of data analyses.

2.3 Assessment of Exposure of Benthic Invertebrates to Chemicals of Potential Concern

To facilitate assessment of risks to the benthic invertebrate community, the sediment chemistry data that were collected during the Phase I and Phase II sampling programs were compiled and summarized. More specifically, summary statistics were calculated for each reach of the study area, including the number of samples collected (n), mean and standard deviation, geometric mean, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles (i.e., these statistics were calculated on $\log_e$ transformed data, based on the assumption that the underlying data were lognormally distributed), and range for each COPC. To facilitate comparisons among reaches, these data were further summarized for each AOC, and for the reference areas. These AOC-specific data summaries included the same statistics that were calculated for the various reaches. Because previous studies have demonstrated that normalization of contaminant concentrations to the variables that are thought to influence bioavailability [e.g., total organic carbon (TOC), grain size, AVS; Barrick *et al.* 1988; Ingersoll *et al.* 1996; Long *et al.* 1998] does not improve the classification of field-collected sediment samples as toxic or not toxic, contaminant concentrations were expressed on a dry-weight basis.

The pore-water chemistry data that were collected during the Phase II sampling program were also summarized for each AOC in a similar manner (i.e., using the same procedures that were applied to the whole-sediment chemistry data). These AOC-specific data summaries included the same statistics that were calculated for the various reaches.

2.4 Assessment of Effects of Chemicals of Potential Concern on Benthic Invertebrates

In this assessment, exposure of the benthic invertebrate community to COPCs was evaluated using information on the concentrations of contaminants in whole sediments and pore water. As such, it was necessary to compile information on the effects on benthic invertebrate communities associated with exposure to COPCs in these environmental media. Evaluation of the potential effects of sediment-associated contaminants on benthic invertebrate communities necessitated the selection of two types of chemical benchmarks, including:

- Toxicity thresholds for whole sediment; and,
- Toxicity thresholds for pore water.

The chemical benchmarks for assessing whole-sediment chemistry and pore-water chemistry data that were selected for use in the BERA are described in the following sections of this report.

Toxicity Thresholds for Whole Sediment - Numerical benchmarks [including sediment quality guidelines (SQGs), sediment quality criteria, sediment quality objectives, and sediment quality standards] represent useful tools for assessing the quality of freshwater sediments (USEPA 1992; Adams *et al.* 1992; USEPA 1996a; USEPA 1997b; Ingersoll and MacDonald 1999; MacDonald *et al.* 2000b; 2000c). Such benchmarks have been developed by various jurisdictions in North America using a variety of approaches. The approaches that have been selected by individual jurisdictions depend on the receptors that are to be considered (i.e., sediment-dwelling organisms, wildlife, or humans), the degree of protection that is to be afforded, the geographic area to which the values are intended to apply (i.e., site-specific, regional, or national), and their intended uses (i.e., screening tools or remediation objectives).

A review of the literature was conducted to identify chemical benchmarks that are relevant for evaluating the effects of sediment-associated COPCs on the benthic invertebrate community. The results of this review indicated that a variety of SQGs have been established for the protection of benthic invertebrates (Table E2-2). The T_{50} (concentration at which 50% of samples are predicted to be toxic) values established by Field *et al.* (2002) were selected as the whole-sediment chemistry benchmarks for assessing the risks to the benthic invertebrate community posed by exposure to COPCs in whole sediments in the Calcasieu Estuary. If T_{50} values were not available for a COPC, then ERMs (Long *et al.* 1995), PELs (MacDonald *et al.* 1996), or apparent effects threshold (AETs; i.e., amphipod, oyster, or benthic AETs; Barrick *et al.* 1988) were used to establish the whole-sediment chemistry benchmark (i.e., toxicity threshold) used to assess risks to the benthic invertebrate community (Table E2-3). More specifically, the measured concentration of each COPC in each sediment sample was compared to the corresponding whole-sediment chemistry benchmark listed in Table E2-3 in order to help identify preliminary contaminants of concern (COCs) from the list of COPCs.

The potential effects of mixtures of sediment-associated contaminants were evaluated using simple toxic units (TUs) models that have been applied successfully at sites in the United States (MacDonald *et al.* 2000b; USEPA 2000b; Ingersoll *et al.* 2001; Appendix E1). Application of these TUs models was facilitated by calculating mean probable effect concentration-quotients (PEC-Qs) for each sediment sample using the procedures developed by USEPA (2000b). The mean PEC-Qs that corresponded to a 20% and a 50% increase in the incidence of sediment toxicity to freshwater and marine amphipods (i.e., relative to reference samples) were determined and used as toxicity thresholds for assessing whole-sediment chemistry data (i.e., in the analysis of incidence of toxicity). Similarly, the mean PEC-Qs that corresponded to a 10% and a 20% reduction in the survival of freshwater and marine amphipods (i.e., relative to reference samples) were determined and used as toxicity thresholds for assessing whole-sediment chemistry data (i.e., in the analysis of magnitude of toxicity). Sediment samples with mean PEC-Qs in excess of these toxicity thresholds were considered to have contaminant concentrations sufficient to adversely affect the survival, growth, and/or reproduction of benthic invertebrates.

Toxicity Thresholds for Pore Water - Pore water is the water that occupies the spaces between sediment particles and usually accounts for over 50% of the volume of surficial sediments. Because sediment-associated contaminants tend to partition into pore water, this medium can represent an important source of exposure to contaminants for sediment-dwelling organisms (Ingersoll *et al.* 1997). For this reason, pore-water assessments can provide useful information on the potential effects of sediment-associated contaminants, particularly on infaunal species (i.e., those species that routinely reside within the sediment matrix). While such assessments can include several components, pore-water toxicity tests and evaluations of pore-water chemistry represent the central elements of most pore-water assessments (ASTM 2001a). Importantly, interpretation of the pore-water chemistry data that is collected in such assessments is dependent on the availability of suitable benchmarks for assessing pore-water quality.

While a variety of benchmarks for assessing pore-water chemistry are available in the published literature, none were identified that were explicitly intended for assessing effects on the benthic invertebrate community. For this reason, the estimated chronic toxicity thresholds in Tables E2-4a and E2-4b (i.e., chronic WQC; CCCs or equivalent values) were used for assessing pore-water quality conditions (ODEQ 1996; NHDES 1996; Suter and Tsao 1996; USEPA 1996b; NYSDEC 1998; USEPA 1999a; LDEQ 2000). Application of these thresholds for assessing the potential effects of COPCs in pore water on benthos is premised on the assumption that benthic invertebrates would exhibit a similar range of sensitivities to COPCs as the species represented in the underlying toxicological data that was used to generate these thresholds (Di Toro *et al.* 1991). Pore-water samples with concentrations of one or more COPCs in excess of one or more toxicity threshold were considered to have contaminant concentrations sufficient to adversely affect the survival, growth, and/or reproduction of benthic invertebrates.

2.5 Characterization of Risks to the Benthic Invertebrate Community

The characterization of the risks to the benthic invertebrate community consisted of three main steps. First, the nature, severity, and areal extent of risks to the benthic invertebrate community were evaluated using one or more lines of evidence. Next, the substances that are causing or substantially contributing to effects on the benthic invertebrate community were identified (i.e., COCs). Finally, the information on multiple lines of evidence was integrated to evaluate the risks to the benthic invertebrate community associated with exposure to COCs. The methods that were used in each of these steps of the process are described in the following sections.

Evaluation of the Nature, Severity and Areal Extent of Risks - In this assessment, five measurement endpoints were used to evaluate the risks posed to the benthic invertebrate community by exposure to COPCs in the Calcasieu Estuary. These lines of evidence included whole-sediment chemistry, whole-sediment toxicity, pore-water chemistry, pore-water toxicity, and benthic invertebrate community structure. More specifically, all five lines of evidence were used to evaluate the nature of the risks to benthic invertebrates. By comparison, whole-sediment chemistry and whole-sediment toxicity were used to evaluate the magnitude (i.e., severity) of effects on benthic invertebrates. By comparison, whole-sediment chemistry data (i.e., PEC-Qs, which were used to estimate the predicted magnitude of toxicity to freshwater and marine amphipods) were used to conduct a preliminary evaluation of the areal extent of risks to benthic invertebrate communities.

The potential effects of mixtures of sediment-associated contaminants were evaluated using simple TUs models that have been validated using data from other sites (MacDonald *et al.* 2000b, USEPA 2000b, Ingersoll *et al.* 2001; Appendix E1). Application of these TUs models was facilitated by calculating mean PEC-Qs for each sediment sample using the procedures developed by USEPA (2000b). These TUs models were calibrated for use in the Calcasieu Estuary by deriving estuary-specific concentration-response relationships for the survival of freshwater and marine amphipods. These estuary-specific models provide a basis for estimating the incidence of toxicity to amphipods (i.e., % toxic samples) or the magnitude of response (i.e., % survival) using whole-sediment chemistry data (Figures E2-6 to E2-11). Importantly, these models also provided a basis for identifying the mean PEC-Qs that correspond to specific incidences and magnitudes of effects (Table E2-5).

To facilitate characterization of the magnitude and areal extent of risks to the benthic macroinvertebrate community, risks were classified into three categories for each sample, reach, and AOC. More specifically, risks to benthic invertebrates were characterized as low, indeterminate, or high, based on the observed and predicted incidence or magnitude of sediment toxicity. The following criteria for classifying risks were established based on the guidance that was provided by the Calcasieu Estuary Ecological Risk Assessment Advisory Group (MacDonald *et al.* 2000a; 2001).

Low Risks - ecological risks were classified as low if the effects that were observed or predicted to occur within a sample, reach, or an AOC were similar in frequency and/or magnitude to those for selected reference areas (Table E2-5). Such effects were considered to be negligible relative to the maintenance of the structure and/or function of the benthic invertebrate community within a reach or an AOC. Nevertheless, conditions that require attention may exist within portions of a reach or AOC that was classified as having low risks to the benthic invertebrate community. Low risks were indicated by:

- 0 to <20% increase in the observed incidence of toxicity (% toxic samples) to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival);

- 0 to <20% increase in the predicted incidence of toxicity to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival);

- 0 to <10% increase in the observed magnitude of toxicity (% survival) to marine amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival);

- 0 to <10% increase in the predicted magnitude of toxicity to marine amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival);

- 0 to <20% increase in the observed incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival);

- 0 to <20% increase in the predicted incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites, (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca;* endpoint: survival);

- 0 to <10% increase in the observed magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival);

- 0 to <10% increase in the predicted magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival);

- 0 to <20% increase in the observed incidence of toxicity to sea urchins within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 48-h pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization or development);

- 0 to <10% increase in the observed magnitude of toxicity based on the fertilization of sea urchin gametes within a sample, reach, or AOC, relative to the fertilization of sea urchin gametes that was observed at selected reference sites (i.e., based on the results of 60-m pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization);

- 0 to <20% increase in the observed incidence of impairment to the benthic invertebrate community, relative to the incidence of impairment that was observed at selected reference sites (i.e., based on the results of cluster analyses conducted using data on the abundance of pollution indicator species, the abundance of pollution sensitive species, species richness, total abundance, and/or normalized benthic index).

Indeterminate Risks - ecological risks were classified as indeterminate if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were moderately higher in frequency and/or magnitude than those for selected reference areas (Table E2-5). Such effects were considered to be of concern relative to the maintenance of the structure and/or function of the benthic invertebrate community within a reach or an AOC. Although such risks are nontrivial, decisions regarding remediation at individual locations should consider the costs and ecological effects of remedial actions, the potential for natural restoration, and other relevant factors. It is important to note that low or high risks to the benthic invertebrate community could exist within portions of a reach or AOC that was classified as posing indeterminate risks. Indeterminate risks were indicated by:

- 20 to 50% increase in the observed incidence of toxicity to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival).

- 20 to 50% increase in the predicted incidence of toxicity to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival).

- 10 to 20% increase in the observed magnitude of toxicity to marine amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival);

- 10 to 20% increase in the predicted magnitude of toxicity to marine amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival);

- 20 to 50% increase in the observed incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival);

- 20 to 50% increase in the predicted incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites, (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca;* endpoint: survival).

- 10 to 20% increase in the observed magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival).

- 10 to 20% increase in the predicted magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival).

- 20 to 50% increase in the observed incidence of toxicity to sea urchins within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 48-h pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization or development).

- 10 to 20% increase in the observed magnitude of toxicity based on fertilization of sea urchin gametes within a sample, reach, or AOC, relative to the fertilization of sea urchin gametes that was observed at selected reference sites (i.e., based on the results of 60-m pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization).

- 20 to 50% increase in the incidence of impairment to the benthic invertebrate community, relative to the incidence of impairment that was observed at selected reference sites (i.e., based on the results of cluster analyses conducted using data on the abundance of pollution indicator species, the abundance of pollution sensitive species, species richness, total abundance, and/or normalized benthic index).

High Risks - ecological risks were classified as high if the effects that were observed or predicted to occur within a sample, a reach, or an AOC were substantially higher in frequency and/or magnitude than those for selected reference areas (Table E2-5). Such effects were considered to be the highest concern relative to the maintenance of the structure and/or function of the benthic invertebrate community within a reach or an AOC. Reaches or AOCs so designated represent the highest priority areas for remedial action planning. It is important to note that low or indeterminate risks to the benthic invertebrate community could exist within portions of a reach or AOC that was classified as posing high risks. Therefore, any remedial actions that are contemplated within such reaches or AOCs should consider the severity and areal extent of the observed and predicted effects. High risks were indicated by:

- >50% increase in the observed incidence of toxicity (based on % toxic samples) to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival).

- >50% increase in the predicted incidence of toxicity to marine amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita;* endpoint: survival).

- >20% increase in the observed magnitude of toxicity to marine amphipods (based on % survival) within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival).

- >20% increase in the predicted magnitude of toxicity to marine amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*; endpoint: survival).

- >50% increase in the observed incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 28-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival);

- >50% increase in the predicted incidence of toxicity to freshwater amphipods within a reach or AOC, relative to the incidence of toxicity that was predicted for selected reference sites, (i.e., based on whole-sediment chemistry and the mean PEC-Q model for 10-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca;* endpoint: survival).

- >20% increase in the observed magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival).

- >20% increase in the predicted magnitude of toxicity to freshwater amphipods within a sample, reach, or AOC, relative to the lower 95% prediction limit for the predicted survival that was observed at selected reference sites (i.e., based on the results of 10-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; endpoint: survival).

- >50% increase in the observed incidence of toxicity to sea urchins within a reach or AOC, relative to the incidence of toxicity that was observed at selected reference sites (i.e., based on the results of 48-h pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization or development).

- >20% increase in the observed magnitude of toxicity based on the fertilization of sea urchin gametes within a sample, reach, or AOC, relative to the fertilization of sea urchin gametes that was observed at selected reference sites (i.e., based on the results of 60-m pore-water toxicity tests with the sea urchin, *Arbacia punctulata;* endpoint: fertilization).

- >50% increase in the incidence of impairment to the benthic invertebrate community, relative to the incidence of impairment that was observed at selected reference sites (i.e., based on the results of cluster analyses conducted using data on the abundance of pollution indicator species, the abundance of pollution sensitive species, species richness, total abundance, and/or normalized benthic index).

Identification of Contaminants of Concern - The COCs in the Calcasieu Estuary were identified using a step-wise approach. In the first step of this process, the concentrations of COPCs in whole sediments or pore water in each reach of the estuary (i.e., 95th percentile concentrations) were compared to the concentrations of COPCs in whole sediments or pore water from the reference areas (i.e., 95th percentile concentrations; the upper limit of background levels). The substances that occurred in the AOC at concentrations that were a factor of two or greater than the upper limit of background concentrations (i.e., 95th percentile concentrations) in the reference areas were retained as preliminary COCs in whole sediments or pore water. Substances were also retained for further assessment if the 95th percentile concentration could not be calculated for the reference area or if the 95th percentile concentration could not be calculated for one or more reaches in an AOC. In both cases, the substance was designated as an uncertain COC. The substances that were designated as preliminary or uncertain COCs were considered to pose potential incremental risks to the benthic invertebrate community.

In the second step of the process, the estimates of the upper limit of the concentrations of preliminary COCs in whole sediments or in pore water (i.e., 95th percentile concentrations) in each reach of the study area were compared to the corresponding chemical benchmark (Tables E2-3 and E2-4). More specifically, the concentrations of preliminary COCs in whole sediments were compared to the whole-sediment chemistry benchmarks, while the concentrations of preliminary COCs in pore water were compared to the chronic toxicity thresholds summarized in Table E2-4. Substances for which the 95th percentile concentration in whole sediment or in pore-water samples in one or more reaches exceeded the selected benchmark were retained as preliminary COCs relative to the benthic invertebrate community (i.e., the substances for which hazard quotients (HQ) of > 1 were calculated, where HQ = concentration ÷ benchmark). A substance was designated as an uncertain COC if there was no benchmark available for the substance or if the 95th percentile concentration could not be determined for one or more reaches within an AOC (i.e., due to high detection limits).

In the final step of the process, cumulative concentration distribution functions were generated for selected preliminary and uncertain COCs identified in Step 1 and 2 above, using the sediment chemistry data collected in Phase II. More specifically, the matching toxicity and chemistry data were used to develop estuary-wide distribution functions for both toxic and non-toxic sediment samples (i.e., based on the results of the whole-sediment toxicity tests, pore-water toxicity tests, or the benthic invertebrate community structure analyses). Substances were retained as COCs if the cumulative distribution functions for the toxic and non-toxic samples diverged substantially in the upper portion of the concentration range (i.e., the 75th percentile concentration for the effects distribution was a factor of two or more greater than the 75th percentile concentration for the no effects distribution; Long *et al.* 1995).

The bioavailability of COPCs was evaluated using the results of 28-d bioaccumulation tests with the polychaete, *Nereis virens*. More specifically, COPCs were considered to be available to benthic invertebrates if their concentrations in polychaete tissues for an AOC exceeded the 95th percentile concentration that was measured in polychaetes exposed to sediments from reference areas.

Integrated Assessment of the Risks to Benthic Invertebrates using a Weight of Evidence Approach - In this assessment, data from chemical analyses, toxicity tests, and biological surveys were used to characterize risks to benthic invertebrates associated with exposure to COPCs in the Calcasieu Estuary. More specifically, the data on up to five lines of evidence, generated during the RI, were used together to estimate risks to benthic invertebrates exposed to whole sediment and/or pore water in the study area. The first step in this process was to calculate a risk score for each measurement endpoint and each line of evidence. Each measurement endpoint was examined to determine if low, indeterminate or high risks were indicated for each sample. A raw risk score of 0, 1, or 2, was assigned to the measurement endpoint based on the risk classification that was assigned (i.e., low, indeterminate, or high, respectively). Next a total evaluation score (TES; i.e., between 1 - low and 3 - high) was calculated to determine the weight that should be placed on the resultant data. The TES was determined by considering a variety of important attributes of the measurement endpoint. By multiplying the magnitude of the risk (raw risk score) by the weight assigned (TES), it was possible to calculate an endpoint risk score of between zero and six for each measurement endpoint. The risk scores for each measurement endpoint were then averaged to calculate an average risk score for each line of evidence for each sample. The information on multiple lines of evidence was then integrated using a simple arithmetic procedure. That is, the average risk score for the various lines of evidence were averaged to generate a final risk score for the assessment endpoint for each sample. Final risk scores of <2, 2 to 3, and >3 were considered to represent low, indeterminate, and high risks to the benthic invertebrate community respectively.

3.0 Results and Discussion

The assessment the risks to the benthic invertebrate community posed by exposure to the COPCs in the Calcasieu Estuary involved several steps. In the first step of the process, the assessment endpoints, risk questions and testable hypotheses, and measurement endpoints were identified (i.e., in the BPF). Next, the relevant information on sediment quality conditions in the Calcasieu Estuary were collected, evaluated, and compiled. Subsequently, the chemical benchmarks for assessing sediment quality conditions were selected, including effects-based SQGs and toxicity thresholds for pore water. Finally, the risks to the benthic invertebrate community associated with exposure to whole sediment and to pore water from the Calcasieu Estuary were assessed. The results of these evaluations are presented in the following sections of this report.

3.1 Assessment Endpoints

Benthic invertebrates are the animals that live in and on the sediments in freshwater and estuarine ecosystems. The groups of organisms that are commonly associated with benthic communities include protozoa, sponges (i.e., Porifera), coelenterates (such as *Hydra* sp.), flatworms (i.e., Platyhelminthes), bryozoans, aquatic worms (i.e., oligochaetes), crustaceans (such as ostracods, mysids, isopods, decapods, and amphipods), mollusks (such as oysters and clams), and aquatic insects (such as dragonflies, mayflies, stoneflies, true flies, caddisflies, and aquatic beetles). Because benthic invertebrate communities are difficult to study in a comprehensive manner, benthic ecologists often focus on the relatively large members of benthic invertebrate communities, which are known as benthic macroinvertebrates. These organisms are usually operationally defined, for example, as those that are retained on a 0.5 mm sieve.

Benthic invertebrates represent key elements of aquatic food webs because they consume aquatic plants (i.e., such as algae and aquatic macrophytes) and detritus. In this way, these organisms facilitate nutrient and energy transfer to fish, birds, and other organisms that consume aquatic invertebrates. As the goal of this assessment is to determine if contaminated sediments have or are likely to have adversely affected the key functions that are provided by the benthic invertebrate community, the **survival, growth, and reproduction of benthic invertebrates** were identified as the assessment endpoints for this component of the BERA.

3.2 Measurement Endpoints

The benthic invertebrate community represents an essential component of aquatic food webs, providing an important source of food for many species of fish, birds, and mammals. As such, it is important to evaluate the effects of environmental contaminants on this group of ecological receptors. Benthic invertebrates can be exposed to environmental contaminants through direct contact with contaminated surface water, through contact with whole sediments, and through contact with contaminated pore water. Of these, exposure to whole sediments and pore water probably represents the primary routes of exposure for epibenthic and infaunal invertebrate species. For this reason, it is important to evaluate the effects of exposure to whole sediments and pore water on the survival, growth, and reproduction of benthic invertebrates. In this way, it is possible to determine if exposure to whole sediment and/or pore water is likely to adversely affect the key ecosystem functions that are provided by the invertebrate community.

A suite of measurement endpoints was selected to provide the information needed to determine if the benthic invertebrate community is being or is likely to be adversely affected due to exposure to COPCs. First, sediment chemistry data were used to determine if the concentrations of COPCs in Calcasieu Estuary sediments are sufficient to cause or substantially contribute to sediment toxicity. In addition, data on the concentrations of COPCs in pore water were used to determine if sediments are sufficiently contaminated to adversely affect the survival, growth, or reproduction of benthic invertebrates in the Calcasieu Estuary. Finally, the results of whole-sediment toxicity tests with amphipods *(Hyalella azteca* and *Ampelisca abdita)*, pore-water toxicity tests with sea urchins *(Arbacia punctulata)*, and benthic invertebrate community assessments were used to evaluate the effects of contaminated sediments on the benthic invertebrate community. More specifically, survival in the *Ampelisca abdita* test, survival or growth in the *Hyalella azteca* test, and fertilization or development in the sea urchin test were used as indicators of the ability of the benthic invertebrate community to perform key functions. Similarly, benthic invertebrate community structure was used as a direct measure of effects on benthos inhabiting the estuary.

3.3 Risk Questions and Testable Hypotheses

To provide a valid basis for assessing ecological effects, the assessment endpoint needs to be linked to the measurement endpoints by a series of risk questions and/or testable hypotheses. In this study, the investigations to assess the effects of environmental contaminants on the benthic invertebrate community were designed to answer the following risk questions:

- Are the levels of COPCs in whole sediments from the AOCs in the Calcasieu Estuary greater than the levels of COPCs in whole sediments from reference areas and greater than sediment quality benchmarks for the survival, growth, or reproduction of benthic invertebrates?
- Are the levels of COPCs in pore water from the AOCs in the Calcasieu Estuary greater than the levels of COPCs in pore water from reference areas and greater than the chronic toxicity thresholds for survival, growth, or reproduction of benthic invertebrates?

- Is the survival of benthic invertebrates (as indicated by the survival of the amphipods, *Hyalella azteca* and *Ampelisca abdita)* exposed to whole sediments from the Calcasieu Estuary outside the normal range [95% confidence intervals; i.e., <2.5th percentile] for amphipods exposed to reference sediments?
- Is the growth of benthic invertebrates (as indicated by the growth of the amphipod, *Hyalella azteca*) exposed to whole sediments from the Calcasieu Estuary outside the normal range (i.e., 95% confidence interval) for amphipods exposed to reference sediments?
- Is the reproductive success of benthic invertebrates (as indicated by fertilization and embryo development in the sea urchin, *Arbacia punctulata*) exposed to pore water from Calcasieu Estuary sediments outside the normal range (i.e., 95% confidence interval) for benthic invertebrates exposed to pore water from reference sediments?
- Is the incidence of impairment of benthic macroinvertebrate community structure (as indicated by the results of cluster analyses of data on the density of pollution indicator species, density of pollution sensitive species, richness, total abundance of benthic invertebrates, and a normalized macroinvertebrate index of biotic integrity) in Calcasieu Estuary sediments elevated relative to the incidence of impairment for reference sediments?

The linkages between the assessment endpoint and the measurement endpoints are articulated in greater detail in the BPF (Appendix A1).

3.4 Exposure of the Benthic Macroinvertebrate Community to Chemicals of Potential Concern

Exposure is the contact or co-occurrence of a contaminant and a receptor (Suter *et al.* 2000). The exposure assessment is intended to provide an estimate of the magnitude of exposure of receptors to COPCs, over time and space. Both baseline exposure and potential future exposure need to be evaluated during the exposure analysis. For the benthic invertebrate community, contaminated sediment and pore water were considered to be the principal routes of exposure requiring analysis.

In this investigation, exposure of benthic invertebrates to COPCs was evaluated for three AOCs and 14 reaches within the study area. Because many of the COPCs considered in this assessment tend to be relatively persistent in sediments, the data that were collected during Phase I and Phase II of the RI were considered to be equivalent and used to assess both current and near-term future exposure to sediment-associated COPCs.

The data on the chemical composition of whole sediments in the Calcasieu Estuary that were collected during Phase I and Phase II of the RI are presented in Appendix B4. Likewise, the available data on the composition of pore water from Calcasieu Estuary sediments is presented in Appendix B5. The data summaries for each reach include the number of samples collected (n), mean and standard deviation, geometric mean, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles, and range for each COPCs (See Tables E2-6 to E2-24). To facilitate comparisons among AOCs, these data were further summarized for each AOC (including the reference area; Tables E2-25 to E2-28). These AOC-specific data summaries include the number of samples in which the substance was detected, total number of samples for each chemical analyte (i.e., substance), as well as the estimates of distributions and central tendency for each analyte. By comparing the concentrations of COPCs in each AOC to the upper limit of background concentrations [i.e., the 95% upper confidence limit (UCL) for sediment samples from reference areas], it is possible to identify the substances that occur at levels that could pose incremental risks to the benthic invertebrate community, relative to the risk that they pose in reference sediments.

Upper Calcasieu River Area of Concern - Within the UCR AOC, the concentrations of chromium, copper, lead, mercury, methyl mercury, zinc, numerous individual PAHs, total low molecular weight PAHs (LMW-PAHs), total high molecular weight PAHs (HMW-PAHs), total PAHs, various PCB congeners and PCB mixtures (Aroclors), total PCBs, BEHP, di-n-butylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2-dichloroethane, 1,1,1-trichloroethane, numerous PCDDs and PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas by a factor of two or more (Tables E2-29 and E2-30). The concentrations of nickel, Aroclor 1221, PCB 80, dimethylphthalate, 1,2,4-trichlorobenzene, 1,2-dichlorobenzene, and certain PCDDs in UCR sediments were not elevated relative to those in reference areas (Tables E2-29 and E2-30).

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from UCR AOC sediments frequently exceed the levels in pore water from reference sediments. More specifically, the concentrations of the following substances were measured in pore-water samples at levels a factor of two or more above the 95% UCL for reference areas: hydrogen sulfide; various individual PAHs; numerous PCB congeners; and, 1,2,4,5-tetrachlorobenzene (Tables E2-31 and E2-32). As such, these substances occur in pore water from

UCR AOC sediments at levels that could pose an incremental risk to benthic invertebrates (i.e., relative to the risks that they pose in pore water from reference sediments).

Bayou d'Inde Area of Concern - The results of Phase I and II of the RI indicate that certain COPCs occur in sediments from the BI AOC at levels that exceed the 95% UCL for reference sediments. More specifically, the concentrations of chromium, copper, lead, mercury, methyl mercury, nickel, zinc, various individual PAHs, total LMW-PAHs, total HMW-PAHs, total PAHs, all of the PCB congeners and PCB mixtures (i.e., Aroclors), total PCBs, dieldrin, BEHP, di-n-butylphthalate, dimethylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1,1-trichloroethane, 1,2-dichloroethane, all of the PCDDs and PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas by a factor of two or more (Tables E2-33 and E2-34). Insufficient data were available to determine if many other substances occurred at elevated concentrations in the BI AOC (e.g., HCB, HCBD).

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from BI AOC sediments frequently exceed the levels in pore water from reference sediments. More specifically, the concentrations of the following substances were measured in pore-water samples at levels a factor of two or more above the 95% UCL for reference areas: hydrogen sulfide; ammonia; lead (dissolved); nickel (total and dissolved); zinc (total and dissolved); 1,1-biphenyl; various individual PAHs; numerous PCB congeners; total PCBs; aldrin; dieldrin; and, HCB (Tables E2-35 and E2-32). As such, these substances occur in pore water from BI AOC sediments at levels that could pose an incremental risk to benthic invertebrates (i.e., relative to the risks that they pose in pore water from reference sediments).

Middle Calcasieu River Area of Concern - Elevated levels of various COPCs (relative to reference sediments) were measured in sediment samples from the MCR AOC. More specifically, the concentrations of chromium, copper, lead, mercury, methyl mercury, zinc, various individual PAHs, total LMW-PAHs, total HMW-PAHs, total PAHs, all of the PCB congeners and PCB mixtures (i.e., Aroclors), total PCBs, BEHP, di-n-butylphthalate, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,1,1-trichloroethane, 1,2-dichloroethane, various PCDDs/PCDFs, TCDD-TEQs, acetone, and carbon disulfide exceeded the 95% UCL for the reference areas by a factor of two or more (Tables E2-36 to E2-38). The levels of sediment-associated nickel, 1,2,4-trichlorobenzene and 1,2-dichlorobenzene were similar in the UCR AOC and the reference areas (Tables E2-36 and E2-37).

The available pore-water chemistry data indicate that the concentrations of COPCs in pore water from MCR AOC sediments frequently exceed the levels in pore water from reference sediments. More specifically, the concentrations of the following substances were measured in pore-water samples at levels a factor of two or more above the 95% UCL for reference areas: 1,1-biphenyl; all of the individual PAHs; numerous PCB congeners; total PCBs; aldrin; dieldrin; HCB; 1,2,3,4-tetrachlorobenzene; 1,2,4,5-tetrachlorobenzene; and, pentachlorobenzene (Tables E2-39, E2-40, and E2-32). As such, these substances occur in pore water from MCR AOC sediments at levels that could pose an incremental risk to benthic invertebrates (i.e., relative to the risks that they pose in pore water from reference sediments).

Summary - The results of the exposure assessment indicate that a number of COPCs occur in whole-sediment samples and/or pore-water samples from the Calcasieu Estuary at concentrations in excess of the 95% UCLs for the selected reference areas. These substances that occur in Calcasieu Estuary sediments and/or pore water at elevated levels relative to reference conditions include: hydrogen sulfide; ammonia; chromium; copper; lead; mercury; methyl mercury; nickel; zinc; 1,1-biphenyl; individual PAHs; total LMW-PAHs; total HMW-PAHs; total PAHs; PCB congeners and mixtures; total PCBs; aldrin; dieldrin; BEHP; di-n-butylphthalate; dimethylphthalate; HCB; 1,3-dichlorobenzene; 1,4-dichlorobenzene; 1,2,3,4-tetrachlorobenzene; 1,2,4,5-tetrachlorobenzene; pentachlorobenzene; 1,1,1-trichloroethane; 1,2-dichloroethane; PCDDs/PCDFs; TCDD-TEQs; acetone; and, carbon disulfide. As a result, it is concluded that these COPCs occur in whole sediments and/or pore water from Calcasieu Estuary at levels that could pose incremental risks to benthic invertebrate communities. Insufficient data were available to determine if certain other substances (e.g., HCBD) occurred at elevated levels (i.e., relative to reference areas) within the three AOCs.

3.5 Effects of Contaminants of Potential Concern on Benthic Invertebrates

In the analysis of effects, risk assessors determine the nature of toxic effects that are associated with exposure to contaminants and their magnitude as a function of exposure (Suter *et al.* 2000). Information on the effects of environmental contaminants may be acquired from the results of single chemical toxicity tests (e.g., spiked-sediment toxicity tests), ambient media toxicity tests (e.g., the results of toxicity tests conducted using sediments collected from the site under investigation), and/or biological surveys (e.g., benthic invertebrate community assessments). Importantly, the data that are collected during this phase of the assessment should be directly related to the exposure estimates (e.g., if the exposure estimates are based on dry weight concentrations of COPCs, the effects data should describe the responses of receptors to changing dry weight concentrations of that COPC), thereby facilitating characterization of risks to each assessment endpoint.

In this assessment, exposure of the benthic invertebrate community to COPCs was evaluated using information on the concentrations of contaminants in whole sediments and pore water. As such, it was necessary to compile information on the effects on benthic invertebrate communities associated with exposure to COPCs in these environmental media. For whole sediments, numerical SQGs are frequently used to evaluate the effects on benthic invertebrates associated with exposure to COPCs. Such SQGs can be developed using the results of equilibrium partitioning modeling, spiked-sediment toxicity tests, and/or investigations of *in situ* sediment quality conditions (i.e., whole-sediment toxicity tests, whole-sediment chemistry analyses, and/or benthic invertebrate community surveys; Ingersoll *et al.* 1997). To support the selection of toxicity thresholds for whole sediments, numerical SQGs were compiled from various sources for each of the COPCs identified in the BPF (i.e., Barrick *et al.* 1988; Long and Morgan 1990; Long *et al.* 1995; MacDonald *et al.* 1996; USEPA 1997b; CCME 1999; 2001; Field *et al.* 2002). The candidate SQGs are presented in Table E2-2.

The toxicity benchmarks for whole sediments were selected using an hierarchical approach. The concentrations that correspond to a 50% probability of observing acute toxicity to marine amphipods (i.e., T_{50} values; Field *et al.* 2002), based on the results of logistic regression modeling of matching sediment chemistry and sediment toxicity data, were selected preferentially as toxicity benchmarks for whole sediments. If T_{50} values were not available for a COPC, then ERM values (i.e., Long and Morgan 1990; Long *et al.* 1995) or PELs (MacDonald *et al.* 1996; CCME 1999; 2001) were selected. For COPCs for which T_{50} values, ERMs, and PELs were not available, the lower of the AETs for oysters, benthos, or amphipods were selected as the toxicity benchmark for whole sediments. Finally, the sediment quality advisory levels (SQALs) that were developed for the National Sediment Inventory evaluation of sediment quality conditions in the United States (USEPA 1997b) were selected when no other SQGs were available. The selected toxicity benchmarks for whole sediments are presented in Table E2-3.

Importantly, all of the toxicity benchmarks that were selected for assessing whole-sediment chemistry data had a similar narrative intent. That is, the selected toxicity benchmarks were intended to identify the concentrations of sediment-associated COPCs above which adverse effects on sediment-dwelling organisms are likely to occur. Accordingly, COPCs that occurred in sediment samples at concentrations in excess of the toxicity benchmarks for whole sediments were identified as preliminary COCs (i.e., one of the substances that are considered to cause or substantially contribute to sediment toxicity). Because sediment-dwelling organisms are likely to respond to all of the COPCs in whole sediments (i.e., rather than individual COPCs), the effects of sediment-associated COPCs were evaluated using chemical mixture models.

The results of the predictive ability evaluation described in Appendix E1 indicate that all of the candidate chemical mixture (i.e., TUs) models considered substantially under-predict the toxicity of Calcasieu Estuary sediments. For this reason, estuary-specific relationships between concentration and response (i.e., incidence of toxicity or percent survival) were evaluated using the matching sediment chemistry and toxicity data that were generated during the Phase II RI. The results of this evaluation indicated that the incidence of toxicity and the magnitude of toxicity to freshwater amphipods in whole sediments were significantly correlated with mean PEC-Q (i.e., $r^2 = 0.98$, $p<0.001$ and $r^2 = 0.91$, $p<0.01$, respectively; Figure E2-8). While the magnitude of whole-sediment toxicity to marine amphipods was significantly correlated with mean PEC-Q (i.e., $r^2 = 0.82$, $p<0.05$), the relationship between the incidence of toxicity and mean PEC-Q was not as strong (i.e., $r^2 = 0.55$, $p=0.21$; Figure E2-11). The models that were developed using the results of pore-water toxicity tests and benthic invertebrate community assessments were not as robust as the models that were developed based on the results of the whole-sediment toxicity tests and, hence, were not used in the effects assessment. The results of these analyses also demonstrated that acute and chronic toxicity to amphipods occurs in Calcasieu Estuary sediments at relatively low mean PEC-Qs (i.e., similar to the mean PEC-Qs that were determined for the sediment samples from reference areas), suggesting that the mean PEC-Q model does not account for all of the factors that are causing or substantially contributing to sediment toxicity.

For pore water, numerical WQC or water quality standards (WQSs) may be used to evaluate the effects on benthic invertebrates associated with exposure to COPCs. Both acute and chronic WQC are promulgated on a national basis by USEPA (1999a). The acute WQC (which are also termed criteria maximum concentrations - CMCs) are calculated as one-half the final acute value (FAV), which is the fifth percentile of the distribution of 48- to 96-h median lethal concentration (LC_{50}) values or equivalent median effective concentration (EC_{50}) values for each COPC (Suter 1996). The CMCs are intended to represent concentrations of COPCs that would cause less than 50% mortality in 5% of the exposed populations in a relatively brief exposure. By comparison, the chronic WQC (which are also termed criteria continuous concentrations - CCCs) are intended to prevent significant toxic effects in most chronic exposures. The CCCs are calculated by dividing the FAV by the final acute-chronic ratio, which is the geometric mean of at least three ratios of LC_{50} to chronic values from toxicity tests on different families of aquatic organisms (Suter 1996).

The toxicity thresholds for pore water were also selected from the candidate water quality benchmarks using an hierarchical approach. More specifically, the chronic marine WQSs for the state of Louisiana were selected preferentially as toxicity thresholds for pore water (LDEQ 2000). The marine CCCs or the marine ecotox thresholds that were promulgated by USEPA (1996b; 1999a) were selected when Louisiana State WQSs were not available. If marine WQSs, CCC, or ecotox thresholds were not available, then chronic toxicity thresholds were estimated from marine acute toxicity thresholds by assuming an acute-to-chronic ratio of 10 (i.e., the FAV was divided by a factor of 10 to estimate the chronic WQC). Alternatively, marine chronic toxicity thresholds were adopted directly from other jurisdictions or estimated from marine acute toxicity thresholds (NHDES 1996; NYSDEC 1998). Finally, a freshwater Tier II secondary chronic value was selected as the toxicity threshold for pore water if none of the other WQSs or WQC were available. The selected toxicity thresholds for pore water are presented in Tables E2-4a and E2-4b.

The toxicity thresholds for pore water are intended to represent the concentrations of COPCs in aqueous media above which toxicity to sensitive aquatic organisms is likely to occur during chronic exposures. That is, adverse effects are expected on 5% of the populations of aquatic organisms that are exposed to such concentrations for protracted periods. Because they are based on the results of single chemical toxicity tests in water-only exposures, samples in which one or more COPCs exceed the toxicity thresholds for pore water were predicted to be toxic to sensitive aquatic organisms in this investigation. This application of the chronic toxicity thresholds is premised on the assumption that sediment-dwelling organisms exhibit the same range of sensitivities to COPCs as do water-column species. This assumption has been demonstrated to be valid, based on the results of water-only toxicity tests with sediment-dwelling and water-column species (Di Toro *et al.* 1991).

3.6 Characterization of Risks to Benthic Invertebrates

The purpose of risk characterization is to determine if significant effects are occurring or are likely to occur at the site under investigation. In addition, this step of the process is intended to provide the information needed to describe the nature, magnitude, and areal extent of effects on the selected assessment endpoints. Finally, the substances that are causing or substantially contributing to such effects (termed COCs) are identified from the COPCs. This information is generated by integrating the results of the exposure assessment with the results of the effects assessment, with each line of evidence initially considered separately. An evaluation of the uncertainty in the analysis provides a basis for determining the level of confidence that can be placed on these results and for integrating multiple lines of evidence into an overall assessment of risks to the benthic invertebrate community. In the final step of the process, the various lines of evidence were considered together to establish a weight of evidence for assessing risks to the assessment endpoint under consideration.

To support the objectives of the risk characterization process, the results of Phase I and Phase II of the RI were compiled and used to assess risks to the benthic invertebrate community associated with exposure to COPCs in sediment and pore water. Five lines of evidence were examined to determine if sediments in the Calcasieu Estuary pose significant risks to the benthic invertebrate community, including whole-sediment chemistry, pore-water chemistry, whole-sediment toxicity, pore-water toxicity, and benthic invertebrate community structure.

Evaluation of the whole-sediment chemistry data collected during Phase I and Phase II of the RI using the estuary-specific concentration-response models (Appendix E1) indicates that roughly 62% (389 of 624) and 36% (225 of 624) of the sediment samples from the three AOCs have concentrations of COPCs that are sufficient to cause or substantially contribute to sediment toxicity to marine amphipods and freshwater amphipods, respectively (Tables E2-41 and E2-42). By comparison, 69% of the whole-sediment samples (52 of 75 samples) collected from the three AOCs were found to be acutely toxic to marine amphipods (endpoint: survival), while 37% (28 of 75 samples) were found to be chronically toxic to freshwater amphipods (endpoints: survival or growth; Tables E2-43 to E2-44). Although, the concentrations of many analytes in pore water were similar to those that were measured in reference areas, the concentrations of hydrogen sulfide and/or ammonia TUs were sufficient to cause chronic toxicity to sediment-dwelling organisms in 69% (52 of 75 samples) of the pore-water samples collected from Calcasieu Estuary sediments (Table E2-45). The incidence of toxicity within the three AOCs was lower (i.e., 5 of 38 samples; 13%) when the results of pore-water toxicity tests with sea urchins (endpoint: fertilization and development) were considered, however (Table E2-46). Finally, the structure of benthic invertebrate communities was impacted in 69% of the sediment samples (52 of 75 samples) collected in the three AOCs (Table E2-47). When considered together, these five lines of evidence indicate that contaminated sediments are adversely affecting the survival, growth, and reproduction of benthic invertebrates in the Calcasieu Estuary.

3.6.1 Upper Calcasieu River Area of Concern

The UCR includes the portion of the watershed from the saltwater barrier to the Highway 210 bridge, a distance of roughly 12 km (or 15 km, including the loop of the river located south of the saltwater barrier). This portion of the river system consists of several readily identifiable water bodies, including the UCR mainstem from the saltwater barrier to Lake Charles, Lake Charles, Calcasieu Ship Channel from Lake Charles to the Highway 210 bridge, Clooney Island Loop, Contraband Bayou, Coon Island Loop, and Bayou Verdine (Figure E2-2). The areas of interest within the UCR AOC include the Clooney Island Loop, Clooney Island Loop Barge Slip, Coon Island Loop Northeast, and Coon Island Loop Southwest (MacDonald *et al.* 2001). To facilitate assessment of risks to the benthic invertebrate community, the UCR AOC was divided into four main reaches, including:

- Upper Calcasieu River - Mainstem and Calcasieu Ship Channel (i.e., from the saltwater barrier to upstream boundary of the BI AOC; Figure E2-2);
- Clooney Island Loop (i.e., Figure E2-2);
- Contraband Bayou (i.e., from the headwaters to the mouth; Figure E2-2);
- Coon Island Loop (Figure E2-2).

The risks to the benthic invertebrate community posed by exposure to contaminated sediments and contaminated pore water were evaluated for each of these reaches and for the UCR AOC as a whole. Additionally, hot spots with respect to contaminated sediments and pore water were identified when possible.

3.6.1.1 Nature of Effects on the Benthic Invertebrate Community in the Upper Calcasieu River Area of Concern

In total, data on five measurement endpoints were used to determine if adverse effects on the benthic invertebrate community were occurring in the UCR AOC in response to exposure to COPCs, including whole-sediment chemistry, pore-water chemistry, whole-sediment toxicity, pore-water toxicity, and benthic invertebrate community structure. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to benthic invertebrate communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring within the study area and to determine the nature of those effects.

When considered in conjunction with numerical SQGs (i.e., chemical mixture models), whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on benthic invertebrates. The whole-sediment chemistry data collected during Phase I and Phase II of the RI were evaluated using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita*. The results of this evaluation indicate that roughly 21% (31 of 146; Table E2-42) and 55% (80 of 146; Table E2-41) of the sediment samples from the UCR AOC have concentrations of metals, PAHs, and or PCBs that are sufficient to cause or substantially contribute to toxicity to freshwater and marine amphipods, respectively. More specifically, these data demonstrate that sediment quality conditions in the UCR AOC are sufficient to reduce the survival and/or growth of sediment-dwelling organisms only marginally.

The pore-water chemistry data collected during the Phase II RI were compared to the chronic toxicity thresholds (Tables E2-45, E2-48, and E2-49). The results of this evaluation indicate that one or more chronic toxicity thresholds for metals were exceeded in all of the pore-water samples (n=15) collected from the UCR AOC in the Calcasieu Estuary, which is the same as the predicted incidence of toxicity for reference areas (i.e., based on one or more exceedances of the toxicity thresholds for pore water). By comparison, the predicted incidence of toxicity was 62% (18 of 29 samples) when only the concentrations of hydrogen sulfide and ammonia were considered, which is higher than the incidence of toxicity (21%; n=14) that was predicted for reference areas (Table E2-47). Therefore, the concentrations of certain conventional variables in pore water from UCR AOC sediments are sufficient to adversely affect benthic invertebrates.

Based on the results of acute and chronic toxicity tests, it is apparent that whole sediments from in the UCR AOC are adversely affecting the survival and/or growth of benthic invertebrates. Of the 29 whole-sediment samples that were collected from the UCR AOCs, a total of 16 (55%) were found to be acutely toxic to marine amphipods (i.e., survival was lower than the 95% LCL (lower confidence limit) for samples from reference areas; Table E2-43). By comparison, 9 of the 29 whole-sediment samples (31%) from the UCR AOCs were chronically toxic to freshwater amphipods (i.e., survival was lower than the 95% LCL for samples from reference areas; Table E2-44). Consideration of the growth endpoint did not result in the designation of additional samples as toxic to freshwater amphipods in this reach. Because reductions in the survival and/or growth of amphipods has been linked to impaired reproductive success (Swartz *et al.* 1994; USEPA 2000b), it is likely that reproduction of sediment-dwelling species would also be impaired in response to exposure to whole sediments in the UCR AOC. These data demonstrate that sediment quality conditions in the UCR AOC are sufficient to adversely affect the survival and/or growth of benthic invertebrates.

The results of pore-water toxicity tests also provide a basis for assessing the risks to benthic invertebrate communities associated with exposure to COPCs in the Calcasieu Estuary. Overall, 1 of the 15 pore-water samples (7%) from the UCR AOC were toxic to the sea urchin, *Arbacia punctulata*, in short-term toxicity tests (i.e., $\leq$48-h) when gamete fertilization and normal embryo development were considered (Table E2-46). These data suggest that the reproduction of benthic invertebrates exposed to UCR AOC sediments was compromised only rarely in the UCR AOC.

Information on the structure of benthic invertebrate communities in the Calcasieu Estuary was also collected as part of the Phase II RI. The results of cluster analyses of these data indicate that the structure of the benthic invertebrate community was impacted in 15 of the 29 sediment samples (52%) that were collected in the UCR AOCs (Table E2-47). As the incidence of effects on the benthic invertebrate community in the UCR AOC was higher than the incidence of effects that was observed for the reference areas, these data suggest that benthic invertebrate community structure has been altered in the UCR AOC.

When considered together, the five lines of evidence indicate that contaminated sediments in the UCR AOC pose risks to the benthic invertebrate community. More specifically, it is likely that the survival, growth, and reproduction of benthic invertebrates are being adversely affected by exposure to contaminated sediments. Therefore, it is concluded that significant effects on the benthic invertebrate community are occurring in the UCR AOC.

3.6.1.2 Magnitude of Effects on the Benthic Invertebrate Community in the Upper Calcasieu River Area of Concern

The magnitude of the effects on benthic invertebrates exposed to contaminated sediments was evaluated using two lines of evidence, including whole-sediment toxicity and whole-sediment chemistry. Based on the results of acute and chronic toxicity tests (i.e., observed magnitude of toxicity), it is apparent that exposure to whole sediments or pore water from the UCR AOC is associated with a range of responses in sediment-dwelling organisms. Of the 29 whole-sediment samples that were collected from the UCR AOCs, a total of 16 (55%) were found to pose a low risk to marine amphipods (i.e., survival rates of *Ampelisca abdita* were similar to those observed for samples from reference areas; Table E2-50). By comparison, the survival of marine amphipods was reduced by 10 to 20% in four of the samples (14%) and by >20% in nine of the samples (31%) from the UCR AOC (Table E2-50). For *Hyalella azteca*, survival in 28-d toxicity tests was similar to that for reference samples in 24 of 29 whole-sediment samples (83%) from the UCR AOC (Table E2-51). By comparison, the survival of freshwater amphipods was reduced by 10 to 20% in one of the samples (3%) and by >20% in four of the samples (14%) from the UCR AOC (Table E2-51). Of the 15 pore-water samples collected from the UCR AOC, one (7%) had sea urchin fertilization rates that were dissimilar to those that were observed in pore water from reference sediments (Table E2-52); a fertilization rate of 1.2% was reported for sea urchins exposed to this pore-water sample.

The magnitude of toxicity to benthic invertebrate communities was also evaluated using the whole-sediment chemistry data collected during Phase I and Phase II of the RI. More specifically, the predicted magnitude of toxicity was determined for each whole-sediment sample using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita* (Figures E2-8 and E2-11). The results of this evaluation indicate that the concentrations of metals, PAHs, and or PCBs in whole-sediment samples from the UCR AOC (i.e., as indicated by mean PEC-Qs) generally pose a low risk to the benthic invertebrate community. The predicted survival of marine amphipods in 121 of 146 (83%) whole-sediment samples from the UCR AOC was within 10% of the lower limit of the normal range (i.e., 95% confidence intervals) of predicted survival rates for the reference areas (Table E2-53); indeterminate and high risks to the benthic invertebrate community were indicated for 11 (8%) and 14 (10%) whole-sediment samples, respectively, based on predicted magnitude of toxicity to marine amphipods (Table E2-53). By comparison, the predicted magnitude of toxicity to freshwater amphipods indicates that 134 (92%), 8 (5%), and 4 (3%) whole-sediment samples from the UCR AOC pose low, indeterminate, and high risks to the benthic invertebrate community (Table E2-54).

Overall, the information on the observed and predicted magnitude of toxicity to freshwater and marine amphipods indicates that exposure to whole sediments from the UCR AOC generally poses a low risk to the benthic invertebrate community (Tables E2-50, E2-51, E2-53, and E2-54). Nevertheless, the concentrations of COPCs are sufficient to cause or substantially contribute to sediment toxicity in at least 18% of the whole-sediment samples collected from this AOC (n=146). Importantly, the survival of marine or freshwater amphipods was reduced by more than 10% relative to reference in at least 45% of the whole-sediment samples tested from this AOC. These results demonstrate that this AOC has a number of hot spots with respect to sediment contamination and toxicity that may require remedial action.

3.6.1.3 Preliminary Assessment of the Areal Extent of Effects on the Benthic Invertebrate Community in the Upper Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on benthic invertebrate communities in the UCR AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of effects, mean PEC-Qs were calculated for each of the sediment samples (n=146) that were obtained from the UCR AOC. Subsequently, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to marine amphipods and freshwater amphipods (i.e., using the estuary-specific concentration-response models). Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Upper Calcasieu River mainstem reach, Clooney Island Loop reach, Contraband Bayou reach, and Coon Island Loop reach.

Upper Calcasieu River - Mainstem Reach - Whole-sediment chemistry data are available for a total of 49 samples from the Upper Calcasieu River mainstem reach of the UCR AOC. The geometric mean of the mean PEC-Q for these samples was 0.145, suggesting that on average sediment-associated contaminants pose relatively low risks to sediment-dwelling organisms in this reach of the estuary (Tables E2-53 and E2-54). Nevertheless, mean PEC-Qs sufficient to reduce the survival of marine amphipods by 10 to 20% relative to reference areas were observed in three of the 49 sediment samples from this reach (Table E2-53 and E2-54). One of the 49 whole-sediment samples collected from this reach had mean PEC-Qs sufficient to reduce the survival of freshwater amphipods by 10 to 20%, relative to reference conditions (Table E2-54). Sediment samples posing indeterminate or high risks to sediment-dwelling organisms were obtained in the eastern portion of Lake Charles and in the wetland area on the western side of Lake Charles (Figures E2-12a to E2-13b). The results of whole-sediment and pore-water toxicity tests indicate that sediment samples from the Calcasieu River immediately upstream of Lake Charles, the south-east portion of Lake Charles, the Calcasieu River immediately downstream of Lake Charles, and the Calcasieu River downstream of Contraband Bayou were toxic to freshwater or marine amphipods, sea urchins, and/or benthos (Figures E2-14 to E2-18). The benthic invertebrate community was impacted in four of the eight samples from this reach (Figure E2-19).

Clooney Island Loop Reach - For the Clooney Island Loop reach of the UCR AOC, whole-sediment chemistry data are available for a total of 32 samples. The geometric mean of the mean PEC-Q for these samples was 0.140 (Tables E2-53 and E2-54), suggesting that sediment-associated contaminants generally pose relatively low risks to sediment-dwelling organisms in this reach of the river. However, four of the whole-sediment samples from this reach had mean PEC-Qs sufficient to reduce the survival of freshwater or marine amphipods by 10 to 20% relative to reference areas (Tables E2-53 and E2-54). Even lower survival (i.e., >20% reduction relative to reference) of freshwater and marine amphipods was predicted for five of the 32 sediment samples from this reach (Tables E2-53 and E2-54). The locations where contaminated sediments pose indeterminate, or high risks to sediment-dwelling organisms include Clooney Island barge slip, in small embayments in the northern and northeastern portion of the loop, and on the northwestern portion of the loop (Figures E2-12a to E2-13b). The results of whole-sediment toxicity tests confirm that whole-sediment samples from throughout the Clooney Island Loop were toxic to freshwater amphipods, marine amphipods, and/or sea urchins, with the most toxic samples (i.e., lowest survival rates) collected from the Clooney Island barge slip and the Clooney Island Loop immediately south of the Olin Chemicals property, which is situated at the north end of the loop between the Conoco and Lyondell Properties (Figures E2-14 to E2-18). Two of the samples from this reach (n=6) had characteristics consistent with an impacted benthic invertebrate community (Figure E2-19).

Contraband Bayou Reach - Based on the results of chemical analysis of nine whole-sediment samples, it is apparent that on average sediment-associated contaminants pose a low risk to benthic invertebrates in the Contraband Bayou reach of Upper Calcasieu River. A geometric mean of the mean PEC-Q of 0.126 was calculated for the reach (Tables E2-53 and E2-54). Application of the concentration-response relationships for both freshwater and marine amphipods to the whole-sediment chemistry data suggests that two of the nine samples collected in this reach pose an indeterminate or high risk to benthic invertebrates (Tables E2-53 and E2-54). The samples with elevated mean PEC-Qs were collected downstream of the Prien Lake Road bridge and nearby the Port of Lake Charles (Figures E2-12a to E2-13b). None of the six samples tested from Contraband Bayou were found to be toxic to freshwater amphipods, marine amphipods and/or sea urchins (Figures E2-14 to E2-18). However, the benthic invertebrate community was impacted in all of the samples from this reach (n=6; Figure E2-19).

Coon Island Loop Reach - Among the four reaches in the UCR AOC, the 56 sediment samples from the Coon Island Loop had the lowest geometric mean of the mean PEC-Qs (0.086; Tables E2-53 and E2-54). As such, risks to the benthic invertebrate community were generally classified low within this reach.

Nevertheless, the whole-sediment chemistry data that are available for this reach indicate that the concentrations of COPCs are sufficient to pose a high risk and an indeterminate risk to benthic invertebrates in seven and five of the 56 sediment samples, respectively (Tables E2-53 and E2-54). The hot spots with respect to sediment contamination (i.e., as indicated by the mean PEC-Q) include the mouth of BV, the northwest portion of Coon Island Loop, and the southeast portion of Coon Island Loop (Figures E2-12a to E2-13b). The results of whole-sediment and pore-water toxicity tests confirm that sediment samples from these locations were toxic to freshwater amphipods, marine amphipods, and/or sea urchins (Figures E2-14 to E2-18). The benthic invertebrate community was impacted in three of the nine samples from this reach (Figure E2-19).

In summary, sediments within the UCR AOC are generally of sufficient quality to support the normal survival, growth, and reproduction of benthic invertebrates. Overall, the mean PEC-Qs calculated for the sediment samples collected from this AOC have chemical characteristics that are similar to those that were measured in the reference areas. Additionally, the incidence and magnitude of toxicity to freshwater and marine amphipods were similar to those that were observed for reference areas. Nevertheless, a number of hot spots with respect to sediment contamination were identified within the UCR AOC, with the highest risks to benthic invertebrates occurring in the Clooney Island barge slip, Lake Charles, Upper Calcasieu River, the northern and northeastern portions of Clooney Island Loop, portions of Contraband Bayou, the mouth of Bayou Verdine, Coon Island Loop northwest, and Coon Island Loop southeast.

3.6.1.4 Contaminants of Concern in the Upper Calcasieu River Area of Concern

Following the assessment of risks to the benthic invertebrate community, it is important to identify the factors that are causing or substantially contributing to adverse effects on sediment-dwelling organisms. In this document, the substances that occur in UCR AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of benthic invertebrates are termed COCs. The COCs in the UCR AOC, relative to the potential for adversely affecting benthic invertebrate communities, were identified by comparing the concentrations of COPCs in whole sediments or pore water to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined to confirm that there was concordance between chemical concentrations and one or more biological responses for each COC identified.

Based on the results of the exposure assessment, a total of 86 substances or groups of substances occurred in whole sediments from the UCR AOC at levels a factor of two or more higher than the 95% UCL for reference sediments (Table E2-30). Subsequent screening against benchmarks for whole-sediment chemistry revealed that the majority of these substances represent preliminary COCs relative to the benthic invertebrate community (Table E2-55). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data or benthic invertebrate community data confirmed that the following substances are considered to be COCs in the UCR AOC (Tables E2-56 to E2-59; Figures E2-20 to E2-56): chromium; copper; lead; mercury; zinc; numerous individual PAHs; total LMW-PAHs; total HMW-PAHs; total PCBs; BEHP; and, TCDD-TEQs (Table E2-59). Historic and/or ongoing sources of these substances are known to occur in the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represented COCs, including: methyl mercury; various individual PAHs; Aroclor 1254; numerous PCB congeners; aldrin; dieldrin; certain phthalates; HCB; HCBD; various chlorinated benzenes; 1,2-dichloroethane; various PCDDs and PCDFs; acetone; and, carbon disulfide.

Data on the concentrations of COPCs in pore-water samples also provides important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table E2-32), and to the toxicity thresholds for pore water (Table E2-60), and in the effects and no effects distributions (based on the results of the sea urchin toxicity test; Table E2-61) indicates that none of the substances considered should be identified as COCs in pore water (Table E2-62). However, hydrogen sulfide should be considered to be a COC since the results of whole-sediment toxicity tests with the amphipod, *Ampelisca abdita*, suggest that it is contributing to sediment toxicity and because pore water samples were manipulated prior to testing in a manner that could alter hydrogen sulfide concentrations.

The substances that occur in whole-sediment or pore-water samples at concentrations above those in reference areas, above the selected benchmarks, and show concordance with the biological response data, represent the COCs relative to effects on benthic invertebrates. In the UCR AOC, the COCs include: hydrogen sulfide; chromium; copper; lead; mercury; zinc; numerous individual PAHs; total LMW-PAHs; total HMW-PAHs; total PCBs; BEHP; and, TCDD-TEQs. The results of 28-d bioaccumulation tests with the polychaete, *Nereis virens*, confirm that the following substances have accumulated to elevated levels in the tissues of benthic invertebrates (i.e., mean concentrations are factor of two or more higher than those in reference areas) and, hence, are bioavailable Calcasieu Estuary sediments (Table E2-63): nickel; chrysene; various PCB congeners; diethylphthalate; BEHP; HCBD; and, virtually all of the PCDDs and PCDFs.

3.6.2 Bayou d'Inde Area of Concern

Bayou d'Inde is one of the major tributaries to the Calcasieu River (Figure E2-3). From its headwaters near Sulphur, Louisiana, Bayou d'Inde flows in a southeasterly direction some 16 km to its confluence with the Calcasieu Ship Channel (or roughly 11 km from the I-10 bridge to the mouth). Over that distance, Bayou d'Inde is joined by several tributaries, the largest of which is Maple Fork. The lower portions of the bayou are characterized by hydraulic connections (i.e., channels that connect the wetlands to the bayou) with a great deal of off-channel wetland habitat, the largest of which is the Lockport Marsh. The areas of interest within the BI AOC include Lower Bayou d'Inde and Middle Bayou d'Inde (MacDonald *et al.* 2001). To facilitate assessment of risks to the benthic invertebrate community, the BI AOC was divided into five reaches, including:

- Upper Bayou d'Inde (i.e., from the headwaters to the Highway 108 bridge; Figure E2-3);
- Middle Bayou d'Inde (i.e., from the Highway 108 bridge to the confluence with PPG Canal; Figure E2-3);
- Lower Bayou d'Inde (i.e., the mainstem from the the confluence with PPG Canal to the confluence with the Calcasieu River; Figure E2-3);

- PPG Canal (i.e., from the headwaters to the confluence with Bayou d'Inde; Figure E2-3); and,
- Lockport Marsh (i.e., the wetland areas located near the mouth of Bayou d'Inde, but excluding the Lower Bayou d'Inde mainstem; Figure E2-3).

The risks to benthic invertebrate community posed by exposure to contaminated sediments and contaminated pore water were evaluated for each of these reaches and for the BI AOC as a whole. Additionally, hot spots with respect to contaminated sediments and pore water were identified when possible.

3.6.2.1 Nature of Effects on the Benthic Invertebrate Community in the Bayou d'Inde Area of Concern

In total, data on five measurement endpoints were used to determine if adverse effects on the benthic invertebrate community were occurring in the BI AOC in response to exposure to COPCs, including whole-sediment chemistry, pore-water chemistry, whole-sediment toxicity, pore-water toxicity, and benthic invertebrate community structure. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to benthic invertebrate communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring or are likely to be occurring within the study area and to determine the nature of those effects.

When considered in conjunction with numerical SQGs, whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on benthic invertebrates. The whole-sediment chemistry data collected during Phase I and Phase II of the RI were evaluated using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita*. The results of this evaluation indicate that roughly 47% (148 of 315; Table E2-42) and 68% (214 of 315; Table E2-41) of the sediment samples from the BI AOCs have concentrations of metals, PAHs, and or PCBs that are sufficient to cause or substantially contribute to toxicity to freshwater and marine amphipods, respectively. More specifically, these data demonstrate that sediment quality conditions in the BI AOC are sufficient to reduce the survival and/or growth of sediment-dwelling organisms.

The pore-water chemistry data collected during the Phase II RI were compared to the chronic toxicity thresholds (Tables E2-45, E2-48, and E2-49). The results of this evaluation indicate that one or more chronic toxicity thresholds for metals were exceeded in all of the pore-water samples (n= 15) collected from the BI AOC; the predicted incidence of pore-water toxicity was also 100% for the samples from the reference areas (Tables E2-45, E2-48, and, E2-49; based on one or more exceedances of the toxicity thresholds for pore water). However, when only the concentrations of hydrogen sulfide and ammonia were considered, the predicted incidence of pore-water toxicity in Bayou d'Inde was 87% (n=31; compared to 21% for the reference areas; Table E2-45). Therefore, the concentrations of certain conventional variables in pore water from BI AOC sediments are sufficient to adversely affect benthic invertebrates.

Based on the results of acute and chronic toxicity tests, it is apparent that whole sediments from the BI AOC are adversely affecting the survival and/or growth of benthic invertebrates. Of the 31 whole-sediment samples that were collected from the BI AOCs, a total of 28 (90%) were found to be acutely toxic to marine amphipods (i.e., survival was lower than the 95% LCL for samples from reference areas; Table E2-43). By comparison, 15 of the 31 whole-sediment samples (48%) from the BI AOCs were chronically toxic to freshwater amphipods (i.e., survival was lower than the 95% LCL for samples from reference areas; Table E2-44). The incidence of toxicity to freshwater amphipods would have been higher had the results for the growth endpoint also been considered in the analysis. Because the reductions in the survival and growth of amphipods have been linked to impaired reproductive success (Swartz *et al.* 1994; USEPA 2000b), it is likely that reproduction of sediment-dwelling species would also be impaired in response to exposure to contaminated sediments in the BI AOC. These data demonstrate that sediment quality conditions in the BI AOC are sufficient to adversely affect the survival and/or growth of benthic invertebrates.

The results of pore-water toxicity tests also provide a basis for assessing the risks to benthic invertebrate communities associated with exposure to COPCs in the Calcasieu Estuary. Overall, 2 of the 15 pore-water samples (13%) from the BI AOC were toxic to the sea urchin, *Arbacia punctulata*, in short-term toxicity tests (i.e., $\leq$48-h) when gamete fertilization and normal embryo development were considered (Table E2-46). While these data suggest that sediment quality conditions sufficient to adversely affect the reproduction of benthic invertebrates do not occur throughout the BI AOC, such effects are likely to occur in certain locations within the AOC.

Information on the structure of benthic invertebrate communities in the Calcasieu Estuary was also collected as part of the Phase II RI. The results of cluster analyses of these data indicate that the structure of the benthic invertebrate community was impacted in 26 of the 31 sediment samples (84%) that were collected in the BI AOC

(Table E2-47; compared to 36% for samples from reference areas), indicating that benthic invertebrate community structure was impacted throughout the BI AOC.

When considered together, the five lines of evidence indicate that contaminated sediments in the BI AOC pose risks to the benthic invertebrate community. That is, it is likely that the survival, growth, and reproduction of benthic invertebrates are being adversely affected by exposure to contaminated sediments. Therefore, it is concluded that significant effects on the benthic invertebrate community are occurring in the BI AOC.

3.6.2.2 Magnitude of Effects on the Benthic Invertebrate Community in the Bayou d'Inde Area of Concern

The magnitude of the effects on benthic invertebrates exposed to contaminated sediments was evaluated using two lines of evidence, including whole-sediment toxicity and whole-sediment chemistry. Based on the results of acute and chronic toxicity tests, it is apparent that exposure to whole sediments or pore water from the BI AOC is associated with a range of responses in sediment-dwelling organisms. Of the 31 whole-sediment samples that were collected from the BI AOCs, a total of three (10%) were found to pose a low risk to marine amphipods (i.e., survival rates of *Ampelisca abdita* were similar to those observed for samples from reference areas; Table E2-50). By comparison, the survival of marine amphipods was reduced by 10 to 20% in seven of the samples (23%) and by >20% in 21 of the samples (68%) from the BI AOC (Table E2-50). For *Hyalella azteca*, survival in 28-d toxicity tests was similar to that for reference samples in 20 of 31 whole-sediment samples (65%) from the BI AOC (Table E2-51). By comparison, the survival of freshwater amphipods was reduced by 10 to 20% in three of the samples (10%) and by >20% in eight of the samples (26%) from the BI AOC (Table E2-51). Of the 15 pore-water samples collected from the BI AOC, two (13%) had sea urchin fertilization rates that were dissimilar to those that were observed in pore water from reference sediments (Table E2-52).

The magnitude of toxicity to benthic invertebrate communities was also evaluated using the whole-sediment chemistry data collected during Phase I and Phase II of the RI. More specifically, the predicted magnitude of toxicity was determined for each whole-sediment sample using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita* (Figures E2-8 and E2-11). The results of this evaluation indicate that the concentrations of metals, PAHs, and or PCBs in whole-sediment samples from the BI AOC (i.e., as indicated by mean PEC-Qs) generally pose an indeterminate risk to the benthic invertebrate community. The predicted survival of marine amphipods in 132 of 315 (42%) whole-sediment samples from the BI AOC was within 10% of the lower limit of the normal range (i.e., 95% confidence intervals) of predicted survival rates for the reference areas (Table E2-53). However, indeterminate and high risks to the benthic invertebrate community were indicated for 33 (10%) and 150 (48%) whole-sediment samples, respectively, based on predicted magnitude of toxicity to marine amphipods (Table E2-53). By comparison, the predicted magnitude of toxicity to freshwater amphipods indicates that 201 (64%), 73 (23%), and 41 (13%) whole-sediments sample from the BI AOC pose a low, indeterminate, and high risk to the benthic invertebrate community (Table E2-54).

Overall, the information on the observed and predicted magnitude of toxicity to freshwater and marine amphipods indicates that exposure to whole sediments from the BI AOC poses variable risks to the benthic invertebrate community (Tables

E2-50, E2-51, E2-53, and E2-54). Additionally, the concentrations of COPCs are sufficient to pose a high risk to freshwater or marine amphipods in 13 to 48% of the sediment samples analyzed from this AOC (n=315), depending on the toxicity test considered (Table E2-53 and E2-54). Importantly, the survival of marine or freshwater amphipods was reduced by more than 10% relative to reference in 35 to 90% of the whole-sediment samples tested from this AOC, depending on the toxicity test considered (Table E2-50 and E2-51). These results demonstrate that sediment contamination and associated toxicity pose unacceptable risks to benthic invertebrate communities throughout much of this AOC.

3.6.2.3 Preliminary Assessment of the Areal Extent of Effects on the Benthic Invertebrate Community in the Bayou d'Inde Area of Concern

A preliminary assessment of the areal extent of adverse effects on benthic invertebrate communities in the BI AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of effects, mean PEC-Qs were calculated for each of the sediment samples (n=315) that were obtained from the BI AOC. Subsequently, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to marine amphipods, freshwater amphipods, and sea urchins (i.e., using the estuary-specific concentration-response model). Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial. The reaches that were considered in this analysis included Upper Bayou d'Inde, Middle Bayou d'Inde, Lower Bayou d'Inde mainstem, Lockport Marsh, and PPG Canal.

Upper Bayou d'Inde Reach - Whole-sediment chemistry data are available for a total of 53 samples from the Upper Bayou d'Inde reach of the BI AOC. The geometric mean of the mean PEC-Q for these samples was 0.265, suggesting that on average sediment-associated contaminants pose relatively low to indeterminate risks to sediment-dwelling organisms in this reach of the estuary (Tables E2-53 and E2-54). Mean PEC-Qs sufficient to reduce the survival of freshwater or marine amphipods by 10 to 20% relative to reference areas were observed in 9 (17%) of the 53 sediment samples from this reach (Tables E2-53 and E2-54). Contaminated sediments pose an indeterminate risk to sediment-dwelling organisms immediately downstream of the CitCon facility (i.e., roughly 1 km upstream of the Highway 108 bridge: Figures E2-57a to E2-58b). The mean PEC-Qs calculated for 19 of the 53 sediment samples indicated a high risk to freshwater or marine amphipods (Tables E2-53 and E2-54); the highest risk locations were located between the CitCon facility and the Highway 108 bridge (Figures E2-57a to E2-58b). The results of whole-sediment and pore-water toxicity tests indicate that the whole-sediment samples from Upper Bayou d'Inde immediately south of the Highway I-10 bridge and those from upstream of the Highway 108 bridge were toxic to freshwater amphipods, marine amphipods and/or to sea urchins (Figures E2-59 to E2-63). Benthic invertebrate communities were also impacted in these areas (Figure E2-64).

Middle Bayou d'Inde Reach - For the Middle Bayou d'Inde reach of the BI AOC, whole-sediment chemistry data are available for a total of 93 samples. The geometric mean of the mean PEC-Q for these samples is 0.369, suggesting that on average sediment-associated contaminants pose indeterminate to high risks to sediment-dwelling organisms in this reach of the estuary (Tables E2-53 and E2-54). Thirty-eight (41%) of the whole-sediment samples from this reach had mean PEC-Qs sufficient to reduce the survival of freshwater or marine amphipods by 10 to 20% relative to reference areas (Tables E2-53 and E2-54). Even lower survival (i.e., >20% reduction relative to reference) of freshwater or marine amphipods was predicted for 71% sediment samples from this reach (Tables E2-53 and E2-54). Contaminated sediments pose a high risk to sediment-dwelling organisms throughout this reach of the BI AOC (Figures E2-57a to E2-58b). The results of whole-sediment and pore-water toxicity tests indicate that samples from the stations located immediately downstream of the Highway 108 bridge and in Maple Fork were toxic to freshwater amphipods, marine amphipods, and/or to sea urchins (Figures E2-59 to E2-63). Benthic invertebrate communities were also impacted in these areas (Figure E2-64).

Lower Bayou d'Inde Reach - Based on the results of chemical analysis of 38 whole-sediment samples, it is apparent that on average sediment-associated contaminants generally pose a low risk to benthic invertebrates in the mainstem portion of Lower Bayou d'Inde. A geometric mean of the mean PEC-Q of 0.198 was calculated for the reach (Tables E2-53 and E2-54). Application of the concentration-response relationships for both freshwater and marine amphipods to the whole-sediment chemistry data suggests that six (16%) of the samples collected in this reach pose an indeterminate risk to benthic invertebrates (Tables E2-53 and E2-54). Eleven (29%) of the samples collected from this reach of the BI AOC had mean PEC-Qs sufficient to pose a high risk to sediment-dwelling organisms, with the sediments posing the highest risks collected within 1.5 km of the mouth of the PPG Canal (Figures E2-57a to E2-58b). All of the samples (n=3) collected from this reach were found to be toxic to freshwater amphipods, marine amphipods, and/or to sea urchins (Figures E2-59 to E2-63). Benthic invertebrate communities were impacted at two of the three locations sampled within this reach (Figure E2-64).

Lockport Marsh Reach - A total of 125 sediment samples were collected from the Lockport Marsh portion of Lower Bayou d'Inde; the geometric mean of the mean PEC-Qs was 0.229 for these samples. Risks to the benthic invertebrate community were generally classified as being low within this reach (Tables E2-53 and E2-54). The whole-sediment chemistry data that are available for this reach indicate that the concentrations of COPCs are sufficient to pose a high risk and an indeterminate risk to benthic invertebrates in 51 (41%) and 19 (15%) sediment samples, respectively (Tables E2-53 and E2-54). The hot spots with respect to sediment contamination (i.e., as indicated by the mean PEC-Q) occurred throughout much of Lockport Marsh, with the levels of risk lowest in the areas located closest to the Calcasieu Ship Channel (Figures E2-57a to E2-58b). The results of whole-sediment toxicity tests confirm that all of the sediment samples from Lockport Marsh are toxic to freshwater amphipods, marine amphipods, and/or to sea urchins (Figures E2-59 to E2-63). The benthic invertebrate community was impacted in most of the samples collected within this reach (i.e., 9 of 12; Figure E2-64).

PPG Canal Reach - Fewer sediment samples were collected from PPG Canal (n=6) than were collected from the other reaches within the BI AOC. When the concentrations of metals, PAHs, and PCBs were considered, these samples had the lowest levels of contamination among the five reaches of the BI AOC (i.e., the geometric mean of the mean PEC-Qs was 0.187; Tables E2-53 and E2-54). While the risks posed to the benthic invertebrate community were generally considered to be low (i.e., based on the average levels of contamination), one of the samples had sufficient levels of contamination to pose an indeterminate risk to benthic invertebrates (Tables E2-53 and E2-54). In addition, three of the samples were considered to pose a high risk to the benthic invertebrate community (Figures E2-57a to E2-58b). Based on the results of whole-sediment toxicity tests with freshwater or marine amphipods, and/or sea urchins, four of these six samples were found to be toxic to sediment-dwelling organisms (Figures E2-59 to E2-63). The benthic invertebrate community was impacted in all the samples from this reach (Figure E2-64).

In summary, sediments within the BI AOC generally pose variable risks to benthic invertebrate communities. The mean PEC-Qs calculated for the sediment samples collected from this AOC were higher than those that were measured in the reference areas. Additionally, the incidence and magnitude of toxicity to freshwater and marine amphipods were elevated, relative to those that were observed for reference areas. Importantly, a number of hot spots with respect to sediment contamination were identified within the BI AOC, with the highest risks to benthic invertebrates occurring in portions of Upper Bayou d'Inde in the vicinity of the Highway 108 bridge, Maple Fork, PPG Canal, the Lower Bayou d'Inde mainstem, and Lockport Marsh. As such, the survival, growth, and reproduction of benthic invertebrates is likely to be impaired throughout much of BI AOC, particularly in those areas located downstream of the CitCon facility (i.e., the lower 8 km of Bayou d'Inde and associated tributaries).

3.6.2.4 Contaminants of Concern in the Bayou d'Inde Area of Concern

Following the assessment of risks to the benthic invertebrate community, it is important to identify the factors that are causing or substantially contributing to adverse effects on sediment-dwelling organisms. In this document, the substances that occur in BI AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of benthic invertebrates are termed COCs. The COCs in the BI AOC, relative to the potential for adversely affecting benthic invertebrate communities, were identified by comparing the concentrations of COPCs in whole sediments or pore water to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined to confirm that there was concordance between chemical concentrations and biological responses.

Based on the results of the exposure assessment, a total of 93 substances or groups of substances occurred in whole sediments from the BI AOC at levels above the 95% UCL for reference sediments (Table E2-34). Subsequent screening against benchmarks for whole-sediment chemistry revealed that the majority of these substances represent preliminary COCs relative to the benthic invertebrate community (Table E2-64). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data or benthic invertebrate community data confirmed the following substances represent COCs in the BI AOC (Tables E2-56 and E2-65; Figures E2-20 to E2-56): chromium; copper; lead; mercury; zinc; various individual PAHs; total PCBs; aldrin; dieldrin; BEHP; HCB; HCBD; and, TCDD-TEQs (Table E2-65). Historic and/or ongoing sources of these substances are known to occur in the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represented COCs, including: methyl mercury; various individual PAHs; Aroclor 1254; all of the PCB congeners; certain phthalates; various chlorinated benzenes; chlorinated ethanes; PCDDs/PCDFs; acetone; and, carbon disulfide.

Data on the concentrations of COPCs in pore-water samples also provides important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table E2-32), and to the toxicity thresholds for pore water (Table E2-60), and in the effects and no effects distributions (based on the results of the sea urchin toxicity test; Table E2-61) indicates that hydrogen sulfide, total nickel, and total zinc should be considered to be COCs in pore water (Table E2-66). The results of 28-d bioaccumulation tests with the polychaete, *Nereis virens*, confirm that the following substances have accumulated to elevated levels in the tissues of benthic invertebrates (i.e., mean concentrations are factor of two or more higher than those in reference areas) and, hence, are bioavailable in BI AOC sediments (Table E2-63): nickel; various PCB congeners; diethylphthalate; HCBD; and, all of the PCDDs and PCDFs measured.

3.6.3 Middle Calcasieu River Area of Concern

The Middle Calcasieu River comprises the portion of the watershed from the Highway 210 bridge to the outlet of Moss Lake (a distance of roughly 12 km), excluding Bayou d'Inde (Figures E2-4a and E2-4b). The primary physiographic features in this portion of the study area include the Calcasieu Ship Channel, Prien Lake, the original Calcasieu River channel, and Moss Lake. For this assessment, the Indian Wells Lagoon and Bayou Olsen were also included in the Middle Calcasieu River study area. The areas of interest within the MCR AOC include South Prien Lake and the Indian Wells Lagoon outflow (MacDonald *et al.* 2001). To facilitate assessment of risks to the benthic invertebrate community, the MCR AOC was divided into five reaches, including:

- Middle Calcasieu River - Mainstem (i.e., Calcasieu Ship Channel and the old river channel, to the outlet of Moss Lake, excluding the portion of the channel south of Prien Lake; Figures E2-4a and E2-4b);
- Prien Lake and the upper old river channel (Figure E2-4a);
- Indian Wells Lagoon (Figure E2-4a);
- Bayou Olsen (i.e., from the headwaters to the mouth; Figure E2-4b); and,
- Moss Lake (i.e., excluding the Calcasieu Ship Channel; Figure E2-4b);.

The risks to benthic invertebrate community posed by exposure to contaminated sediments and contaminated pore water were evaluated for each of these reaches and for the MCR AOC as a whole. Additionally, hot spots with respect to contaminated sediments and pore water were identified when possible.

3.6.3.1 Nature of Effects on the Benthic Invertebrate Community in the Middle Calcasieu River Area of Concern

In total, data on five measurement endpoints were used to determine if adverse effects on the benthic invertebrate community were occurring in the MCR AOC in response to exposure to COPCs, including whole-sediment chemistry, pore-water chemistry, whole-sediment toxicity, pore-water toxicity, and benthic invertebrate community structure. These data also provided a basis for assessing the nature of the effects that are occurring or are likely to be occurring to benthic invertebrate communities. Both the presence of conditions sufficient to cause or substantially contribute to effects (i.e., predicted incidence of toxicity) and occurrence of specific types of effects (i.e., observed incidence of toxicity) were used to determine if effects are occurring within the study area and to determine the nature of those effects.

When considered in conjunction with numerical SQGs, whole-sediment chemistry data provide a basis for evaluating the effects of contaminated sediments on benthic invertebrates. The whole-sediment chemistry data collected during Phase I and Phase II of the RI were evaluated using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita*. The results of this evaluation indicate that roughly 29% (48 of 163; Table E2-42) and 59% (96 of 163; Table E2-41) of the sediment samples from the MCR AOCs have concentrations of metals, PAHs, and or PCBs that are sufficient to cause or substantially contribute to toxicity to freshwater and marine amphipods, respectively. These data demonstrate that sediment quality conditions in the MCR AOC are sufficient to reduce the survival and growth of sediment-dwelling organisms.

The pore-water chemistry data collected during the Phase II RI were compared to the chronic toxicity thresholds (Tables E2-45, E2-48, and E2-49). The results of this evaluation indicate that the chronic toxicity thresholds for total ammonia or hydrogen sulfide were exceeded in 47% of the samples (i.e., 7 of 15) that were collected from the MCR AOC (based on one or more exceedances of the toxicity thresholds for pore water; Table E2-45). For the other COPCs (i.e., metals or organic substances), one or more chronic toxicity thresholds were exceeded in all of the pore-water samples (n=8) collected from the MCR AOC of the Calcasieu Estuary (Tables E2-48 and E2-49). Therefore, the concentrations of certain conventional variables in pore water from MCR AOC sediments are sufficient to adversely affect benthic invertebrates.

Based on the results of acute and chronic toxicity tests, it is apparent that whole sediments from the MCR AOC are adversely affecting the survival and/or growth of benthic invertebrates. Of the 15 whole-sediment samples that were collected from the MCR AOCs, a total of eight (53%) were found to be acutely toxic to marine amphipods (i.e., survival was lower than the 95% LCL for samples from reference areas; Table E2-43). By comparison, four of the 15 whole-sediment samples (27%) from the MCR AOCs were chronically toxic to freshwater amphipods (i.e., survival or growth was lower than the 95% LCL for samples from reference areas; Table E2-44). Because the reductions in the survival or growth of amphipods have been linked to impaired reproductive success (Swartz *et al.* 1994; USEPA 2000b), it is likely that reproduction of sediment-dwelling species would also be impaired in response to exposure to contaminated sediments in the MCR AOC. These data demonstrate that sediment quality conditions in the MCR AOC are sufficient to adversely affect the survival and/or growth of benthic invertebrates.

The results of pore-water toxicity tests also provide a basis for assessing the risks to benthic invertebrate communities associated with exposure to COPCs in the Calcasieu Estuary. Overall, two of the eight pore-water samples (25%) from the MCR AOC were toxic to the sea urchin, *Arbacia punctulata*, in short-term toxicity tests (i.e., ≤48-h) when gamete fertilization and normal embryo development were considered (Table E2-46). These data suggest that sediment quality conditions sufficient to adversely affect the reproduction of benthic invertebrates occur in portions of the MCR AOC.

Information on the structure of benthic invertebrate communities in the Calcasieu Estuary was also collected as part of the Phase II of the RI. The results of cluster analyses of these data indicate that the structure of the benthic invertebrate community was impacted in six of the 15 sediment samples (40%) that were collected in the MCR AOC (Table E2-47). When considered on a per reach basis, the incidence of effects on the benthic invertebrate community in the MCR AOC was similar to or lower than the incidence of effects that was observed for the reference areas in all reaches except Indian Wells Lagoon (100%; n=3) and Bayou Olsen (60%; n=5).

When considered together, the five lines of evidence indicate that contaminated sediments in the MCR AOC pose risks to the benthic invertebrate community. More specifically, it is likely that the survival, growth, and reproduction of benthic invertebrates are being adversely affected by exposure to contaminated sediments. Therefore, it is concluded that significant effects on the benthic invertebrate community are occurring in the MCR AOC.

3.6.3.2 Magnitude of Effects on the Benthic Invertebrate Community in the Middle Calcasieu River Area of Concern

The magnitude of the effects on benthic invertebrates exposed to contaminated sediments was evaluated using two lines of evidence, including whole-sediment toxicity and whole-sediment chemistry. Based on the results of acute and chronic toxicity tests, it is apparent that exposure to whole sediments or pore water from the MCR AOC is associated with a range of responses in sediment-dwelling organisms. Of the 15 whole-sediment samples that were collected from the MCR AOC, a total of eight (53%) were found to pose a low risk to marine amphipods (i.e., survival rates of *Ampelisca abdita* were similar to those observed for samples from reference areas; Table E2-50). By comparison, the survival of marine amphipods was reduced by 10 to 20% in three of the samples (20%) and by >20% in four of the samples (27%)

from the MCR AOC (Table E2-50). For *Hyalella azteca*, survival in 28-d toxicity tests was similar to that for reference samples in 12 of 15 whole-sediment samples (80%) from the MCR AOC (Table E2-51). By comparison, the survival of freshwater amphipods was reduced by 10 to 20% in one of the samples (7%) and by >20% in two of the samples (13%) from the MCR AOC (Table E2-51). Of the eight pore-water samples collected from the MCR AOC, two (25%) had sea urchin fertilization rates that were dissimilar to those that were observed in pore water from reference sediments (Table E2-52); both samples were considered to pose a high risk to benthic invertebrates.

The magnitude of toxicity to benthic invertebrate communities was also evaluated using the whole-sediment chemistry data collected during Phase I and Phase II of the RI. More specifically, the predicted magnitude of toxicity was determined for each whole-sediment sample using estuary-specific concentration-response models for the amphipods, *Hyalella azteca* and *Ampelisca abdita* (Figures E2-8 and E2-11). The results of this evaluation indicate that the concentrations of metals, PAHs, and or PCBs in whole-sediment samples from the MCR AOC (i.e., as indicated by mean PEC-Qs) generally pose a low risk to the benthic invertebrate community. The predicted survival of marine amphipods in 138 of 163 (85%) whole-sediment samples from the MCR AOC was within 10% of the lower limit of the normal range (i.e., 95% confidence intervals) of predicted survival rates for the reference areas (Table E2-53); indeterminate and high risks to the benthic invertebrate community were indicated for 7 (4%) and 18 (11%) whole-sediment samples, respectively, based on predicted magnitude of toxicity to marine amphipods (Table E2-53). By comparison, the predicted magnitude of toxicity to freshwater amphipods indicates that 154 (94%), 2 (1%), and 7 (4%) whole-sediment samples from the MCR AOC pose a low, indeterminate, and high risk to the benthic invertebrate community (Table E2-54).

Overall, the information on the observed and predicted magnitude of toxicity to freshwater and marine amphipods indicates that exposure to whole sediments from the MCR AOC generally poses a low risk to the benthic invertebrate community (Tables E2-50, E2-51, E2-53, and E2-54). Nevertheless, the concentrations of COPCs are sufficient to cause or substantially contribute to sediment toxicity in at least 15% of the whole-sediment samples collected from this AOC (n=163). Importantly, the survival of marine or freshwater amphipods was reduced by more than 10% relative to reference in at least 47% of the whole-sediment samples tested from this AOC (i.e., 7 of 15 samples). These results demonstrate that this AOC has a number of hot spots with respect to sediment contamination and toxicity that may require remedial action.

3.6.3.3 Preliminary Assessment of the Areal Extent of Effects on the Benthic Invertebrate Community in the Middle Calcasieu River Area of Concern

A preliminary assessment of the areal extent of adverse effects on benthic invertebrate communities in the MCR AOC was conducted using the whole-sediment chemistry data that were collected in Phase I and Phase II of the RI. To support this evaluation of the spatial distribution of effects, mean PEC-Qs were calculated for each of the sediment samples (n=163) that were obtained from the MCR AOC. Subsequently, each sediment sample was classified into one of three categories (i.e., low, indeterminate, or high), based on the risk that it posed to marine amphipods, freshwater amphipods, and sea urchins (i.e., using the estuary-specific concentration-response model). Then, these data were compiled on a reach by reach basis and mapped using ArcView/Spatial Analyst software. The reaches that were considered in this analysis included the Middle Calcasieu River mainstem, Prien Lake and the upper old river channel, Indian Wells Lagoon, Bayou Olsen, and Moss Lake.

Middle Calcasieu River - Mainstem Reach - Whole-sediment chemistry data are available for a total of 76 samples from the Middle Calcasieu River mainstem reach of the MCR AOC. The geometric mean of the mean PEC-Q for these samples is 0.147, suggesting that an average sediment-associated contaminants pose relatively low risks to sediment-dwelling organisms in this reach of the estuary (Tables E2-53 and E2-54). Nevertheless, mean PEC-Qs sufficient to reduce the survival of marine amphipods by 10 to 20% relative to reference areas were observed in five (7%) of the 76 sediment samples from this reach (Table E2-53). In addition, two (3%) of the 76 whole-sediment samples collected from this reach had mean PEC-Qs sufficient to reduce the survival of freshwater amphipods by 10 to 20%, relative to reference conditions (Table E2-54). Sediment-associated contaminants pose higher risks (i.e., >20% reduction in predicted survival) to marine amphipods at seven locations (9%) in this reach (Table E2-53). The locations where contaminated sediments pose an indeterminate or high risk to sediment-dwelling organisms principally included the vicinity of the Citgo and W.R. Grace properties on the Calcasieu Ship Channel (Figures E2-65a, E2-65b, E2-68a, and E2-69a). No samples were collected to support toxicity testing of whole sediments or benthic invertebrate community structure assessment in this reach.

Prien Lake and Upper Old River Channel Reach - For the Prien Lake and upper old river channel reach of the MCR AOC, whole-sediment chemistry data are available for a total of 49 samples. The geometric mean of the mean PEC-Q for these samples is 0.114, suggesting that on average sediment-associated contaminants generally pose relatively low risks to sediment-dwelling organisms in this reach of the estuary (Tables E2-53 and E2-54). None of the whole-sediment samples from this reach had mean PEC-Qs sufficient to reduce the survival of freshwater or marine amphipods by 10 to 20% relative to reference areas (Tables E2-53 and E2-54). Lower survival (i.e., >20% reduction relative to reference) of freshwater and marine amphipods was predicted for two (4%) sediment samples from this reach (Tables E2-53 and E2-54). The locations where contaminated sediments pose a high risk to sediment-dwelling organisms include: the west central portion of Prien Lake and the northern portion of the old river channel (Figures E2-65a, E2-65b, E2-66a and E2-66b). The results of whole-sediment toxicity tests indicate that whole-sediment samples from the southwest portion of Indian Bay (at the terminus of Henderson Road) and in the most southerly portion of the old river channel (Figures E2-67a, E2-68a, E2-69a, and E2-70a) were toxic to freshwater amphipods or marine amphipods. Benthic invertebrate community structure was not impacted in any of the samples collected from this reach (n=4; Figure E2-72a).

Indian Wells Lagoon Reach - Based on the results of chemical analysis of 10 whole-sediment samples, it is apparent that sediment-associated contaminants generally pose a high risk to benthic invertebrates in the Indian Wells Lagoon reach of Middle Calcasieu River. A geometric mean of the mean PEC-Q of 1.15 was calculated for the reach, which is the highest for any reach of the estuary (Tables E2-53 and E2-54). Application of the concentration-response relationships for both freshwater and marine amphipods to the whole-sediment chemistry data suggests that none of the samples collected in this reach pose an indeterminate risk to benthic invertebrates (Tables E2-53 and E2-54). However, eight (80%) of the samples pose a high risk to freshwater or marine amphipods (Tables E2-53 and E2-54), with sediment samples from the central and northerly portions of the lagoon posing the highest risks to benthic invertebrate communities (Figures E2-65a, E2-65b, E2-66a, and E2-66b). All of the samples (n=3) collected from Indian Wells Lagoon were found to be toxic to freshwater amphipods, marine amphipods, and/or sea urchins (Figures E2-67a, E2-68b, E2-69a, E2-70a, and E2-71a). Benthic invertebrate community structure was impacted in all of the samples collected from this reach (n=3; Figure E2-72a).

Bayou Olsen Reach - Based on the available whole-sediment chemistry data, Bayou Olsen had among the lowest geometric mean of the mean PEC-Qs (0.116) for the MCR AOC. As such, risks to the benthic invertebrate community were, on average, classified as low within this reach (Tables E2-53 and E2-54). Evaluation of these whole-sediment chemistry data on a sample by sample basis indicates that the concentrations of COPCs are likely to pose low risks to benthic invertebrates (Tables E2-53 and E2-54; Figures E2-65c and E2-66c). However, the results of whole-sediment toxicity tests demonstrate that 40% of the sediment samples (n=5) from Bayou Olsen are toxic to freshwater or marine amphipods (Figures E2-67b, E2-68b, E2-69b, and E2-70b; Tables E2-43 and E2-44). Likewise, benthic invertebrate community structure was impacted in most (i.e., 3 of 5) samples collected from this reach (Figure E2-72b). These results suggest that stressors other than metals, PAHs and PCBs are causing or substantially contributing to the effects that have been observed in this reach.

Moss Lake Reach - For Moss Lake, whole-sediment chemistry data are available for 17 sediment samples. The geometric mean of the mean PEC-Qs that were calculated for these samples (0.159) suggests that sediments within this reach of the MCR AOC generally pose relatively low risks to benthic invertebrate communities (Tables E2-53 and E2-54). Nevertheless, the concentrations of sediment-associated contaminants are sufficient to pose indeterminate and high risks in two (12%) and one (6%) of the 17 sediment samples, respectively (Tables E2-53 and E2-54; Figures E2-65c and E2-66c). Of the three whole-sediment samples that were tested for this reach, one was found to be acutely toxic to marine amphipods, freshwater amphipods, or sea urchins (Figures E2-67b, E2-68b, E2-69b, E2-70b, and E2-71b). None of the samples from this reach had impacted benthic invertebrate community structure (n=3; Figure E2-72b).

In summary, sediments within the MCR AOC are generally of sufficient quality to support the normal survival, growth, and reproduction of benthic invertebrates. Overall, the mean PEC-Qs calculated (0.154) indicate that the sediment samples collected from this AOC have chemical characteristics that are similar to those that were measured in the reference areas. However, roughly 15% of the sediment samples from the MCR AOC have contaminant concentrations sufficient to pose indeterminate or high risks to freshwater and marine amphipods. The hot spots with respect to sediment contamination are largely associated with the Middle Calcasieu River mainstem (i.e., western shoreline) and the Indian Wells Lagoon reaches of the MCR AOC.

3.6.3.4 Contaminants of Concern in the Middle Calcasieu River Area of Concern

Following the assessment of risks to the benthic invertebrate community, it is important to identify the factors that are causing or substantially contributing to adverse effects on sediment-dwelling organisms. In this document, the substances that occur in MCR AOC sediments at concentrations that are sufficient to cause or substantially contribute adverse effects on the survival, growth, or reproduction of benthic invertebrates are termed COCs. The COCs in the MCR AOC, relative to the potential for adversely affecting benthic invertebrate communities, were identified by comparing the concentrations of COPCs in whole sediments and pore water to the concentrations of those substances in reference sediments and to the selected benchmarks for those substances. Additionally, the distributions of the effects and no effects data for each biological endpoint were examined to confirm that there was concordance between chemical concentrations and biological responses.

Based on the results of the exposure assessment, a total of 86 substances or groups of substances occurred in whole sediments from the MCR AOC at levels above the 95% UCL for reference sediments (Table E2-37). Subsequent screening against benchmarks for whole-sediment chemistry revealed that the majority of these substances represent preliminary COCs relative to the benthic invertebrate community (Table E2-67). Divergence of the cumulative effects and no effects distributions compiled using the matching sediment chemistry and laboratory toxicity data or benthic invertebrate community data confirmed that the substances that represent COCs in the MCR AOC include (Tables E2-56 to E2-58; Figures E2-20 to E2-56): chromium; copper; lead; mercury; zinc; numerous individual PAHs; total LMW-PAHs; total HMW-PAHs; total PAHs; total PCBs; aldrin; BEHP; HCB; and TCDD-TEQs (Table E2-68). Historic and/or ongoing sources of all of these substances are known to occur in the estuary (see BPF for more information on the sources of these COCs). Insufficient information was available to determine if many other substances represented COCs, including methyl mercury, various individual PAHs, Aroclor 1254, various PCB congeners, dieldrin, certain phthalates, HCBD and other chlorinated benzenes, 1,2-dichloroethane, PCDDs/PCDFs, acetone, and carbon disulfide.

Data on the concentrations of COPCs in pore-water samples also provides important information for identifying COCs. Comparison of the measured concentrations of COPCs in pore water to the concentrations in pore water from reference sediments (Table E2-32), and to the toxicity thresholds for pore water (Table E2-60), and in the effects and no effects distributions (based on the results of the sea urchin toxicity test; Table E2-61) indicates that hydrogen sulfide, 1-methylnaphthalene, benz(a)anthracene, and benzo(a)pyrene should be considered to be COCs in pore water (Table E2-69).

The substances that occur in whole-sediment or pore-water samples at concentrations above those in reference areas, above the selected benchmarks, and show concordance with the biological response data, represent the COCs relative to effects on benthic invertebrates. In the MCR AOC, the COCs include hydrogen sulfide, chromium, copper, lead, mercury, zinc, numerous individual PAHs, total LMW-PAHs, total HMW-PAHs, total PAHs, total PCBs, aldrin, BEHP, HCB, and TCDD-TEQs. The results of 28-d bioaccumulation tests with the polychaete, *Nereis virens*, confirm that the following substances have accumulated to elevated levels in the tissues of benthic invertebrates (i.e., mean concentrations are factor of two or more higher than those in reference areas) and, hence, are bioavailable in MCR AOC sediments (Table E2-63): chromium, nickel, various PCB congeners, BEHP, and certain PCDDs and PCDFs.

4.0 Uncertainty Analysis

There are a number of sources of uncertainty in assessments of risk to the benthic invertebrate community, including uncertainties in the conceptual model, in the exposure assessment, and in the effects assessment. As each of these sources of uncertainty can influence the estimations of risk, it is important to describe and, when possible, quantify the magnitude and direction of such uncertainties. The purpose of this section is to evaluate uncertainty in a manner that facilitates attribution of the level of confidence that can be placed in the assessments conducted using the various lines of evidence. Accordingly, the uncertainties associated with the assessment of risks to benthic invertebrate communities are described in the following sections.

4.1 Uncertainties Associated with the Conceptual Model

The conceptual model is intended to define the linkages between stressors, potential exposure, and predicted effects on ecological receptors. As such, the conceptual model provides the scientific basis for selecting assessment and measurement endpoints to support the risk assessment process. Potential uncertainties arise from lack of knowledge regarding ecosystem functions, failure to adequately address spatial and temporal variability in the evaluations of sources, fate, and effects, omission of stressors, and overlooking secondary effects (USEPA 1998). The types of uncertainties that are associated with the conceptual model that links contaminant sources to effects on the benthic invertebrate community include those associated with the identification of COPCs, environmental fate and transport of COPCs, exposure pathways, receptors at risk, and ecological effects. Of these, the identification of exposure pathways probably represents the primary source of uncertainty in the conceptual model.

In this assessment, it was assumed that exposure to whole sediments and pore water represents the most important pathways for exposing benthic invertebrate communities to COPCs (i.e., as the benthic invertebrates associated with benthic habitats likely play the most important role key ecological functions and contaminant concentrations are likely to be highest in these media types). However, benthic invertebrates may also be exposed to COPCs in the water column. As such, risks to the benthic invertebrate community could be under-estimated if this pathway resulted in significant exposure of benthic benthos to COPCs.

4.2 Uncertainties Associated with the Exposure Assessment

The exposure assessment is intended to describe the actual or potential co-occurrence of stressors with receptors. As such, the exposure assessment identifies the exposure pathways and the intensity and extent of contact with stressors for each receptor or group of receptors at risk. There are a number of potential sources of uncertainty in the exposure assessment, including measurement errors, extrapolation errors, and data gaps.

In this assessment, two types of measurements were used to evaluate exposure of benthic invertebrate communities to COPCs: chemical analyses of environmental media (i.e., whole sediment and pore water); and, toxicity tests conducted using indicator species. Relative to the pore-water and whole-sediment chemistry data, analytical errors and descriptive errors represent potential sources of uncertainty. Three approaches were used to address concerns relative to these sources of uncertainty. First, analytical errors were evaluated using information on the accuracy, precision, and detection limits that are generated to support the Phase I and Phase II sampling programs. The results of this analysis indicated that most of the data used in this assessment met the project data quality objectives (see Appendix B1 for more details). Second, all data entry, data translation, and data manipulations were audited to assure their accuracy. Data auditing involved 10% number-for-number checks against the primary data source initially, increasing to 100% number-for-number checks if significant errors were detected in the initial auditing step. Finally, statistical analyses of resultant data were conducted to evaluate data distributions, identify the appropriate summary statistics to generate, and evaluate the variability in the observations. As such, measurement errors in the pore-water and whole-sediment chemistry data are considered to be of minor importance and are unlikely to substantially influence the results of the risk assessment.

The treatment of whole-sediment or pore-water chemistry data has the potential to influence the results of the BERA. In particular, the treatment of less than detection limit data can effect the results of the exposure assessment and the risk characterization. A number of investigators have evaluated the implications of applying various procedures for estimating the concentrations of COPCs from less than detection limit data (Gaskin *et al.* 1990; Porter and Ward 1991; El-Shaawari and Esterby 1992; Clarke and Brandon 1994). While there is no consensus on which data censoring method should be used in various applications, the simplest methods tend to be used most frequently, including deletion of non-detect values or substitution of a constant, such as zero, the detection limit, or one-half the detection limit (USACE 1995).

To address the need for guidelines for statistical treatment of less than detection limit data, the USACE (1995) conducted a simulation study to assess the performance of 10 methods for censoring data. The results of that investigation indicated that no single data censoring methods works best in all situations. Accordingly, United

States Army Corps of Engineers (USACE) recommended a variety of methods depending on the proportion of the data that requires censoring, the distribution and variance of the data, and the type of data transformation. For data sets for which a low to moderate proportion of the data require censoring, substitution of the detection limit is generally the preferred methods (i.e., to optimize statistical power and control type I error rates). However, as the proportion of the data that requires censoring and the coefficient of variation of the data increase, statistical power is better maintained by substituting of one-half the detection for the less than detection limit data, particularly for lognormally distributed and transformed data. Substitution of zero or other constants was also recommended for a variety of circumstances. Overall, it was concluded that simple substitution methods work best to maintain power and control error rates in statistical comparisons of chemical concentration data (USACE 1995).

In this analysis, decisions regarding the treatment of less than detection limit data were made by considering the recommendations that have emerged from previous investigations in the context of their potential effects on the results of the BERA. Including all of the whole-sediment or pore-water chemistry data that were collected in the Calcasieu Estuary RI, roughly 80% of the data required censoring prior to data analysis. To minimize the potential effects of the less than detection limit data on the results of the BERA, none of the less than detection limit data for which the detection limits were greater than the corresponding toxicity thresholds for whole-sediment or pore-water chemistry (i.e., benchmarks) were used in the exposure analysis. Consistent with the guidance developed by USACE (1995), one-half of the detection limit was substituted for all of the other less than detection limit data. This procedure facilitated the estimation of distributions of the concentrations of COPCs and eliminated the potential for identifying significant risks based on less than detection limit data.

Selection of an alternate procedure for treating the less than detection limit data has the potential for influencing the results of the BERA. For example, substitution of zero for less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentile concentrations would likely have been lower than the estimates developed for the exposure assessment). Likewise, substitution of the detection limit for the less than detection limit data would have altered the distributions of the COPC concentration data for the three AOCs and the reference area (i.e., the estimated 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentile concentrations would likely have been higher than the estimates developed for the exposure assessment). Although the influence of these alternate methods on the estimate of the 75^{th} or 95^{th} percentile concentration would likely have been relatively minor, their selection could have influenced the identification of COCs for one or more AOCs. However, neither the nature, magnitude, nor areal distribution of risks to benthic invertebrate communities was affected by the selection of data treatment methods. As such, the potential impact of the methods that were selected for treating less than detection limit data on the results of the BERA are considered to be minor.

Data gaps also represent a source of uncertainty in the assessments of exposure for aquatic receptors. For example, the available data on the chemical composition of pore waters were limited to 50 samples (i.e., compared to over 500 for whole sediments). As such, it is difficult to assess exposure of benthic invertebrate communities to pore water in certain reaches of the Calcasieu Estuary. Although this data gap is significant, it is mitigated to a large extent by the abundance of data on whole-sediment chemistry.

4.3 Uncertainties in the Effects Assessment

The effects assessment is intended to describe the effects that are caused by stressors, link them to the assessment endpoints, and evaluate how effects change with fluctuations in the levels (i.e., concentrations) of the various stressors. There are several potential sources of uncertainty in the assessment of effects on aquatic receptors, including measurement errors, extrapolation errors, and data gaps.

In this investigation, the effects on benthic invertebrate communities associated with exposure to sediment-associated COPCs were evaluated using several types of information, including toxicity benchmarks for whole-sediment chemistry, toxicity benchmarks for pore water, whole-sediment and pore-water toxicity tests, and estuary-specific concentration-response relationships. Although the toxicity benchmarks are not subject to measurement errors, the toxicity tests are. For this reason, potential measurement errors associated with toxicity tests were also evaluated in the uncertainty analysis. More specifically, the data on negative controls and positive controls were examined to identify potential measurement errors. In addition, the results obtained from samples collected in the reference areas were considered in this analysis. More specifically, a reference envelope approach was used to classify whole-sediment and pore-water samples as toxic or not toxic. Because this approach facilitated the determination of the normal range of responses for samples from reference areas and only samples for which the response was beyond the 95% LCL (i.e., 25^{th} percentile), the probability of incorrectly classifying a not-toxic sample as toxic is roughly 0.025. However, the probability of incorrectly classifying a toxic sample as not-toxic is probably higher. Therefore, application of the reference envelope approach may tend to underestimate risks to the benthic invertebrate community. Because the concentration-response relationships were developed using the whole-sediment chemistry and toxicity data, these models are subject to the same measurement errors that were ascribed to the underlying data. Importantly, the steps that were taken to minimize the potential impacts of measurement errors on the results of the analyses conducted with the whole-sediment chemistry or whole-sediment toxicity data also reduce the uncertainties associated with concentration-response models.

There are several sources of extrapolation errors in the effects assessment for the Calcasieu Estuary BERA. First, the toxicity benchmarks for whole sediments were primarily the concentrations that corresponded to a 50% probability of observing toxicity to marine amphipods. Although these benchmarks were considered to provide a conservative basis for identifying COCs, it is possible that these benchmarks could underestimate the effects of sediment-associated contaminants on more sensitive benthic species. Second, the toxicity benchmarks for pore water are primarily chronic WQC for marine organisms or functionally equivalent values. While these WQCs are intended to be broadly applicable to waterbodies in the United States, their relevance to sediment-dwelling organisms could be questioned. For this reason, Di Toro *et al.* (1991) conducted an evaluation of the applicability of the WQC and concluded that they can be used in sediment quality assessments (e.g., for deriving equilibrium partitioning-based sediment guidelines). Third, indicator species have been used in this investigation to assess the effects of contaminated sediments on the benthic invertebrate community. Uncertainties associated with the application of this approach were evaluated by reviewing the results of sediment quality assessments conducted at other sites. This review indicates that the results of whole-sediment toxicity tests are frequently well correlated with results of assessments of benthic invertebrate community structure (Ingersoll *et al.* 2002; Swartz *et al.* 1994; MacDonald *et al.* 2002; Hayward 2002). In some cases, adverse effects on the benthic invertebrate community have been observed at lower levels of contamination than is the case for the whole-sediment toxicity tests (Hyland *et al.* 2002; MacDonald *et al.* 2002), probably due to the combined effects of physical and chemical stressors. The results of the selected pore-water toxicity tests have also been correlated with adverse effects on sediment-dwelling organisms (Carr *et al.* 1996c). Finally, the concentration-response models that were included in the effects assessment were developed using estuary specific data. In this way, the uncertainties associated with extrapolation of the models between areas was avoided. The use of multiple lines of evidence also minimizes the potential effects of extrapolation errors on the results of the BERA.

Uncertainty in the effects assessment for aquatic receptors is also increased by data gaps. To the extent possible, this source of uncertainty was mitigated by collecting detailed information on the effects of COPCs in the Calcasieu Estuary. In addition, the use of multiple lines of evidence provides a basis for minimizing the influence of data gaps on the effects assessment. Nevertheless, limitations on certain types of data, such as concentration-response data for various benthic species, makes it difficult to fully evaluate the effects of COPC exposures on benthic invertebrate communities. For this reason, the present assessment could over-estimate or under-estimate risks to the benthic invertebrate community.

5.0 Integrated Assessment of Risks to Benthic Invertebrates in the Calcasieu Estuary Using a Weight of Evidence Approach

Information on five lines of evidence and multiple measurement endpoints for each line of evidence was compiled to support the assessment of risks to the benthic invertebrate community associated with exposure to contaminated sediments in the Calcasieu Estuary. The previous sections of this appendix present the information for each of these individual lines of evidence, and interpret that information to evaluate effects on the survival, growth, or reproduction of benthic invertebrates. As such, the previous evaluations were used to provide the information needed to: determine if adverse effects on benthic invertebrates are occurring, or are likely to be occurring, within the Calcasieu Estuary; to evaluate the nature, severity, and areal extent of such effects; and, to identify the substances that are causing or substantially contributing to effects on the benthic invertebrate community.

Each of the lines of evidence that was used in the assessment of risks to benthic invertebrate communities has certain strengths and limitations that influence its application in the risk assessment process. For this reason, an uncertainty analysis was conducted to evaluate the level of confidence that can be placed in the results of analyses conducted using the individual lines of evidence (Section 4.0). Importantly, the uncertainty associated with the overall assessment of risks can be reduced by integrating information from multiple lines of evidence using a weight of evidence approach (Ingersoll and MacDonald 2002).

Historically, the sediment quality triad approach has been used to evaluate concordance between measures of sediment chemistry, sediment toxicity, and benthic invertebrate community structure. The contingency table presented in Table E2-70 presents eight possible outcomes, based on the correspondence among these three indicators of sediment quality conditions. In this investigation, multiple lines of evidence are available for only a subset of the sediment samples that were collected during the RI. For this reason, it was important to develop a procedure for integrating multiple lines of evidence that could be applied regardless of the number of lines of evidence that were available for a sample.

In this investigation, a simple arithmetic procedure was used to integrate information from multiple lines of evidence. In the first step of this process, the level of confidence (i.e., weight; as quantified by calculating a total evaluation score - TES; Table E2-71) that could be placed in each measurement endpoint was scored from one (low) to three (high) determined based on the following considerations (i.e., adapted from Suter *et al.* 2000):

<u>Conceptual Model:</u>

- *Relevance of Exposure Pathway:* Evidence was given more weight if the exposure pathway examined was the most relevant for assessing the status of the assessment endpoint. For example, exposure of aquatic plants to surface water would be considered to be more relevant than exposure to whole sediments; and,
- *Relevance of the Measurement Endpoint*: Evidence was given more weight if the measurement endpoint provided a direct estimate of the status of the assessment endpoint or if validation studies have demonstrated that measurement endpoint is predictive of the assessment endpoint. For example, measurement of the survival and growth of amphipods is considered to provide direct evidence for evaluating the survival and growth of benthic invertebrates. By comparison, data on the fertilization

and development of sea urchin gametes and embryos (which are pelagic life history stages) is considered to provide less direct evidence for evaluating the reproduction of benthic invertebrates.

Exposure Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on exposure of receptors at risk to COPCs. For example, the level of standardization for surface-water chemistry and whole-sediment chemistry would be high because standard methods were used to collect, handle, transport, store, and analyse samples. By comparison, the level of standardization for pore-water chemistry would be lower because standard methods for collecting and processing samples have not been described;
- *Quality of Data:* Evidence was given more weight if the data were demonstrated to be of high quality. In this context, data quality was evaluated by considering the project data quality objectives and consider such criteria as accuracy, precision, and analytical detection limits;
- *Quantity of Data:* Evidence was given more weight if the sample size was considered to be adequate to characterize conditions within the study area;
- *Level of Temporal Coverage:* Evidence was given more weight if the data encompasses the relevant range of temporal variance of conditions. For example, a single sampling event was considered to evaluate characterize whole-sediment chemistry because such conditions are unlikely to change substantially on a seasonal basis. In contrast, surface-water chemistry is likely to change on daily and seasonal bases, emphasizing the need for more comprehensive data sets to characterize temporal variability; and,

- *Level of Spatial Coverage:* Evidence was given more weight if the data adequately represented the geographic area that was being assessed. In this context, a measurement endpoint was scored high if samples were available from most or all of the areas of concern and associated reaches.

Effects Assessment:

- *Level of Standardization*: Evidence was given more weight if standard methods were available and appropriately implemented to generate the data on the effects associated with exposure of receptors at risk to COPCs. For example, concentration-response relationships were considered to be stronger if the underlying toxicity tests and chemical analyses were conducted using standard methods;
- *Meets Acceptability Criteria*: Evidence was given more weight if the established acceptability criteria for the measurement endpoint were met. For example, toxicity test results were weighted high if the negative and positive control results were with acceptable ranges;
- *Demonstrated Concentration - Response Relationship:* Evidence was given more weight if it demonstrated a relationship between the magnitude of exposure and the effects;
- *Relevance of the Exposure Medium*: Evidence was given more weight if the medium (i.e., surface water, sediment) considered in the assessment was consistent with the mode of exposure for the site medium. For example, the results of pore-water toxicity tests would be less relevant for assessing the status of the aquatic plant community than would the results of surface-water toxicity tests;
- *Level of Field Validation:* Evidence was given more weight if the results of validation studies have demonstrated that the measurement endpoint

provides a reliable basis estimating the status of the assessment endpoint or the status of other measurement endpoints that are predictive of the assessment endpoint.

Consideration of the result that was obtained for each measurement endpoint (e.g., observed incidence of toxicity to marine amphipods, which was score 0, 1, or 2), in conjunction with the weight (i.e., TES) that was assigned to that measurement endpoint (which was scored from 1 to 3), provided a basis for developing a risk score for each measurement endpoint and line of evidence (Section 2.5). Subsequently, a final risk score was calculated by averaging the risk score for each line of evidence.

The final risk score is intended to provide an integrated measure of the risks that contaminated sediments pose to the survival, growth, or reproduction of benthic invertebrate communities in the Calcasieu Estuary. More specifically, the final risk score integrates the results of whole-sediment toxicity tests, pore-water toxicity tests, whole-sediment chemical analyses, pore-water chemical analyses, and benthic invertebrate community analyses into a single parameter. While data for multiple lines of evidence were available for only 100 of the 641 sediment samples that were included in the analysis, the results of statistical analyses of the underlying data indicate that the final risk score is positively correlated with the risk score for whole-sediment chemistry (Figure E2-73). This is important because all five lines of evidence are available for only 50 sediment samples and three lines of evidence are available for only 100 sediment samples.

The concordance between the final risk score and the risk score for whole-sediment chemistry indicates that the final risk scores are likely to be comparable regardless of the number of lines of evidence that were considered in its calculation (i.e., one to five lines of evidence). Overall, 71 of 89 samples (80%) for which multiple lines of evidence were available were classified the same (i.e., as low, indeterminate or high risk) regardless of the number of lines of evidence considered. Nevertheless, it is important to note that final risk scores that are based on whole-sediment chemistry alone could underestimate risks to the benthic invertebrate community in some cases. Roughly 2% (2 of 89) of the samples with low risk scores for whole-sediment chemistry (i.e., <2) had final risk scores that were indicative of high risk to benthic invertebrate communities. Another 13% (12 of 89) of the samples were classified as low risk using the risk score for whole-sediment chemistry, but were classified as indeterminate risk when multiple lines of evidence were considered. Although final risk scores calculated using one line of evidence (i.e., sediment chemistry) may underestimate risks in certain circumstances, they generally provide a reliable basis for estimating risks to the benthic invertebrate community. The results of the integrated assessment of risks to benthic invertebrates in the Calcasieu Estuary are presented in the following sections of this appendix.

5.1 Integrated Assessment of Risks to Benthic Invertebrates in the Upper Calcasieu River Area of Concern

Samples were collected from a total of 146 locations to support an assessment of the risks posed to the benthic invertebrate community associated with exposure to sediment and pore water within the UCR AOC. The results of this assessment indicate that exposure to whole sediments and/or pore water in the UCR AOC generally posed a low risk to benthic invertebrates (i.e., average of the final risk scores of 0.838; n=146). Low final risk scores (i.e., < 2) were calculated for 86% of the locations (i.e., 126 of 146) sampled within this AOC (Table E2-72).

Nevertheless, indeterminate (4%; 6 of 146) or high (10%; 14 of 146) risks to benthic invertebrates were indicated for 14% of the locations that were sampled (i.e., 20 of 146) in the UCR AOC (Table E2-72). Consistent with the results of the analyses of the information on individual lines of evidence, the locations where contaminated sediments posed the highest risks (i.e., relative to the survival, growth, or reproduction of benthic invertebrates) included the eastern portion of Lake Charles, the Clooney Island barge slip, the northeastern and southwestern portions of the Clooney Island Loop, the mouth of Bayou Verdine, and the northern, northwestern, and central portions of Coon Island Loop (Figures E2-74a and E2-74b). Indeterminate risks to the benthic invertebrate community exist in the eastern portion of Lake Charles, in the Calcasieu River downstream of Lake Charles, the eastern portion of Clooney Island Loop, in Contraband Bayou in the vicinity of the Port of Lake Charles, and the central and southern portions of Coon Island Loop (Figures E2-74a and E2-74b).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to benthic invertebrates than are sediments posing low risks (Table E2-73). In addition, this increased sediment toxicity is sufficient to adversely affect the structure of the benthic invertebrate community. Therefore, it is concluded that the survival, growth, or reproduction of benthic invertebrates is being adversely affected by exposure to contaminated sediments in portions of the UCR AOC relative to reference conditions. Because many of the COCs in the UCR AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the benthic invertebrate community unless corrective action is taken to reduce risks in high (and certain indeterminate) risk locations.

5.2 Integrated Assessment of Risks to Benthic Invertebrates in the Bayou d'Inde Area of Concern

Samples were collected from a total of 315 locations to support an assessment of the risks posed to the benthic invertebrate community associated with exposure to sediment and pore water within the BI AOC. The results of this assessment indicate that exposure to whole sediments and/or pore water in the BI AOC posed risks to benthic invertebrates ranging from low to high (i.e., average of the final risk scores of 2.20; n=315). Roughly 49% of the locations that were sampled (i.e., 153 of 315) in this AOC had low final risk scores (i.e., < 2; Table E2-72). However, indeterminate (14%; 44 of 315) or high (37%; 118 of 315) risks to benthic invertebrates were indicated for 51% of the locations (i.e., 162 of 315) in the BI AOC (Table E2-72). Consistent with the results of the analyses of the individual lines of evidence, the locations where contaminated sediments posed the highest risks (i.e., relative to the survival, growth, or reproduction of benthic invertebrates) included Upper Bayou d'Inde from the CitCon property to the Highway 108 bridge, the off-channel wetland and mainstem areas throughout Middle Bayou d'Inde, PPG Canal, the portions of Lockport Marsh closest to PPG Canal, the central portion of the wetland area located east of Lower Bayou d'Inde, and Lower Bayou d'Inde mainstem (Figures E2-75a and E2-75b). Indeterminate risks to the benthic invertebrate community exist in Upper Bayou d'Inde downstream of the I-10 bridge and from the CitCon property to the Highway 108 bridge, the mainstem and off-channel wetland areas throughout Middle Bayou d'Inde, PPG Canal, the central portions of Lockport Marsh and the wetland area located southeast of Lower Bayou d'Inde, and the Lower Bayou d'Inde mainstem (Figures E2-75a and E2-75b).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to benthic invertebrates than are sediments posing low risks (Table E2-73). In addition, this increased sediment toxicity is sufficient to adversely affect the structure of the benthic invertebrate community. Therefore, it is concluded that the survival, growth, or reproduction of benthic invertebrates is being adversely affected by exposure to contaminated sediments in portions of the BI AOC relative to reference conditions. Because many of the COCs in the BI AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the benthic invertebrate community unless corrective action is taken to reduce risks in high (and certain indeterminate) risk locations.

5.3 Integrated Assessment of Risks to Benthic Invertebrates in the Middle Calcasieu River Area of Concern

Samples were collected from a total of 163 locations to support an assessment of the risks posed to the benthic invertebrate community associated with exposure to sediment and pore water within the MCR AOC. The results of this assessment indicate that exposure to whole sediments and/or pore water in the MCR AOC generally posed a low risk to benthic invertebrates (i.e., average of the final risk scores of 0.674; n=163). Eighty-eight percent of the locations that were sampled (i.e., 144 of 163) in this AOC had low final risk scores (i.e., < 2; Table E2-72). Nevertheless, indeterminate (5%; 8 of 163) or high (7%; 11 of 163) risks to benthic invertebrates were indicated for 12% of the locations (i.e., 19 of 163) in the MCR AOC (Table E2-72). Consistent with the results of the analyses of the individual lines of evidence, the locations where contaminated sediments posed the highest risks (i.e., relative to the survival, growth, or reproduction of benthic invertebrates) included the Middle Calcasieu River adjacent to the Citgo property and Indian Wells Lagoon (Figures E2-76a, E2-76b, and E2-76c). Indeterminate risks to the benthic invertebrate community exist in portions of Prien Lake and the upper old river channel, in the Middle Calcasieu River in the vicinity of the Citgo property and the W.R. Grace property, and the central portion of Moss Lake (Figures E2-76a, E2-76b, and E2-76c).

The biological conditions that occur within the three risk categories indicate that sediments posing indeterminate and high risks are more toxic to benthic invertebrates than are sediments posing low risks (Table E2-73). In addition, this increased sediment toxicity is sufficient to adversely affect the structure of the benthic invertebrate community. Therefore, it is concluded that the survival, growth, or reproduction of benthic invertebrates is being adversely affected by exposure to contaminated sediments in portions of the MCR AOC relative to reference conditions. Because many of the COCs in the MCR AOC are considered to be relatively persistent in surficial and deeper sediments and there is little evidence that COC concentrations are decreasing over time (see Table E1-7 of Appendix E1), it is likely that such effects will continue to impact the benthic invertebrate community unless corrective action is taken to reduce risks in high (and certain indeterminate) risk locations.

6.0 Summary and Conclusions

The risks to benthic invertebrate communities posed by exposure to whole sediments and pore water were assessed in the Calcasieu Estuary. In total, information on five lines of evidence was used to determine if the survival, growth, and/or reproduction of benthic invertebrates have been adversely affected or is likely to have been adversely affected by exposure to contaminated sediments in the estuary relative to reference conditions. The five lines of evidence that were considered in the assessment included whole-sediment chemistry, whole-sediment toxicity, pore-water chemistry, pore-water toxicity, and benthic invertebrate community structure.

The results of this assessment indicated that exposure to whole sediment and/or pore water from the Calcasieu Estuary generally posed low risks to benthic invertebrate communities (i.e., risks were classified as low for 68% of the locations sampled (423 of 624) within the three AOCs investigated. However, indeterminate and high risks to the benthic invertebrate community were indicated for 9% (58 of 624) and 23% (143 of 624) of the locations sampled, respectively (Table E2-71). Of the three AOCs considered, the risks to the benthic invertebrate community were highest in Bayou d'Inde, based both on the incidence and magnitude of toxicity (i.e., observed and predicted). Within this AOC, samples from the lower portions of Upper Bayou d'Inde, Middle Bayou d'Inde, PPG Canal, the inner portions of Lockport Marsh, and Lower Bayou d'Inde mainstem posed the highest risks. Although risks to the benthic invertebrate community were generally lower in the UCR AOC and MCR AOC, samples posing a high risk to benthic invertebrates were collected from the northern portions of Clooney Island Loop, the northern portions of Coon Island Loop, the Middle Calcasieu River in the vicinity of the Citgo property, and Indian Wells Lagoon. Risks to the benthic invertebrate community are generally low throughout the reference areas (Figure E2-77). An assessment of the risks posed to benthic invertebrates associated with exposure to COPCs at each of the stations sampled in the Calcasieu Estuary is presented in Tables E2-74 to E2-92.

The results of the biological investigations conducted during the RI indicate that the magnitude of effects tends to increase with increasing risk to the benthic invertebrate community. For example, the survival and/or growth of freshwater and marine amphipods were lower for the locations that were designated as posing indeterminate and high risks than was the case for the locations that were classified as posing low risks to benthic invertebrates (Table E2-72). Likewise, the fertilization of sea urchin gametes was reduced in the samples from locations that were designated as posing indeterminate or high risks to the benthic invertebrate community (Table E2-72). Importantly, the density of pollution indicator (i.e., tolerant) species, the density of pollution sensitive species, species richness, and total abundance of benthic invertebrates were generally lower for the sampling locations that were classified as posing indeterminate and high risks, as compared to the sampling locations that posed low risks to benthic invertebrates (Table E2-72). Together, these results demonstrate that the survival, growth, and/or reproduction of benthic invertebrates have been impaired in response to exposure to contaminated sediments in the Calcasieu Estuary.

The results of this assessment indicated that a number of substances are causing or substantially contributing to adverse effects on the benthic invertebrate community in the Calcasieu Estuary. More specifically, the COCs included:

- Hydrogen sulfide;
- Metals (chromium, copper, lead, mercury, nickel, and zinc);
- PAHs (1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, phenanthrene, total LMW-PAHs,

benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, fluoranthene, indeno(1,2,3-cd)pyrene, pyrene, total HMW-PAHs, and total PAHs);

- PCBs (total PCBs);
- Chlorinated benzenes (HCB and HCBD);
- Phthalates (BEHP);
- Organochlorine pesticides (aldrin and dieldrin); and,
- PCDDs and PCDFs (total 2,3,7,8-TCDD TEQs).

All of these substances occurred in whole-sediment and/or pore-water samples from the Calcasieu Estuary at concentrations in excess of those observed in samples from reference areas and in excess of the selected benchmarks. In addition, the concentrations in the effects distribution were generally higher than the concentrations in the no effects distribution for one or more of the measurement endpoints (e.g., survival of *Ampelisca abdita* in 10-d toxicity tests). Many of these substances or groups of substances also accumulated in the tissues of polychaetes (*Nereis virens*) in 28-d bioaccumulation tests and were shown to be associated with toxicity to amphipods (*Ampelisca abdita)* in toxicity identification evaluations.

7.0 References

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